USING CALUX ASSAY TO SELECT HIGH-TCDD MOTHERS IN SOUTHERN KAZAKHSTAN AND MAP TIME-COURSE OF TCDD ELIMINATION VIA BREAST MILK

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Introduction

In 1994, in the first comprehensive investigation of persistent organochlorine contaminants in a country of the former Soviet Union, we measured congener-specific levels of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs), as well as 19 organochlorine pesticides (OC) in breast milk samples collected using the WHO protocol from first-time mothers ("primiparae") living in southern Kazakhstan[1-3]. High levels (up to 80 pg/g fat) of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were found in breast milk samples from villages in a cotton-growing region in southern Kazakhstan, with TCDD contributing 70-80 % of the I-TEQ[1,2]. In these samples, the I-TEQ arose almost exclusively from two congeners, TCDD and the pentachlorodibenzo-p-dioxin (PeCDD). A follow-up study in 1997 identified a "hot zone" of PCDDs/PCDFs in the region's cotton-growing State Farms[4]. As part of our continuing characterization of this TCDD contamination, we used the CALUX assay to screen and identify breast milk donors with high contaminant levels, and to assay the change in concentration of TEQs in breast milk samples from these donors during the lactational period. We report here on partial results from the time-course study, in which samples were collected from November, 2000-June, 2001.

Materials and Methods

Study design

Breast milk samples were collected in November, 2000 from 75 donors residing in the "hot zone" using the WHO/EURO selection criteria (healthy primiparae with healthy infants 2-8 weeks of age)[5]. The collection procedures (design, exposure assessment questionnaire, informed consent) are described in detail elsewhere[1,2]. Mothers hand-expressed separate milk samples into both chemically-clean 20 ml- and 100 ml-sample collection jars for analysis by CALUX and HRGC/HRMS, respectively. Samples from the 75 first-time mothers were screened for polychlorinated dioxin/furan TEQs using a modified CALUX assay and mothers with high dioxin/furan TEQs were invited to participate in a time-course study. The time-course study collected 5 monthly breast milk samples starting in February, 2001 which were also analyzed by the modified CALUX assay.

Analytical methods

CALUX *Assay.* Xenobiotic Detection Systems, Inc. (XDS), has a patented genetically engineered cell line which contains the firefly luciferase gene under trans-activational control of the aryl

hydrocarbon receptor[10]. The cell line can be used for the detection and relative quantification of PCDDs, PCDFs, and co-planar PCBs when used with a patented sample process procedure[11]. The assay using this cell line is called the Chemical-Activated Luciferase expression, or CALUX, assay.

Using the CALUX assay, breast milk samples were analyzed for dioxin/furan TEQ activity as expressed by the reporter gene assay. The sample extracts in DMSO were suspended in cell culture medium, just prior to dosing on monolayers of H1.L1.6 mouse hepatoma cells that were grown in 96-well culture plates. In addition to the dilution of samples, a standard curve of TCDD-concentrations was assayed (128.8, 64.4, 32.2, 16.1, 8.0, 4.0, 2.0, 1.0, 0.5, and 0.1 ppt of TCDD). The plates were incubated for optimal induction of luciferase activity in a humidified CO_2 incubator. After the incubation, the media was removed and the cells were microscopically observed for viability. The luciferase activity was quantified using the substrate kit of Promega™ (Madison, WI).

Results and Discussion

The time-course study collected samples in November 2000, and 5 monthly samples from February-June, 2001. We evaluated off-loading trends from 13 mothers by plotting dioxin/furan TEQ (pg/g fat) vs. "Days Postpartum". Curve shapes fell into 3 categories: decreasing (N=5), increasing (2), and unclear (6) trends (see Figures 1-3 for examples). The variation in curve shapes may arise from several factors, including (a) different background levels of TCDD contamination for individuals living in the "hot spot" region, (b) different inter-individual variations in uptake and/or offloading rates, and (c) differences in BMI, which may contribute to inter-individual differences in TCDD excretion. Since earlier studies indicated reasonably uniform background (i.e. contamination of the food supply [4,7]), variations in off-loading rates of TCDD seen here may reflect the differences between individuals in the uptake and/or elimination of this chemical.

In an earlier study of breast milk samples (N=21) from this region, GC/MSMS and CALUX results correlated well: TEQs measured in human milk using the CALUX method correlated with I-TEQs calculated using GC/MS/MS data, even with TEQs as low as 1 pg/g wet weight (Spearman correlation coefficient = 0.78)[12]. The present study suggests that CALUX is useful not only as a screening assay in epidemiological studies to select individuals with high TEQ body burdens, but as a quantitative

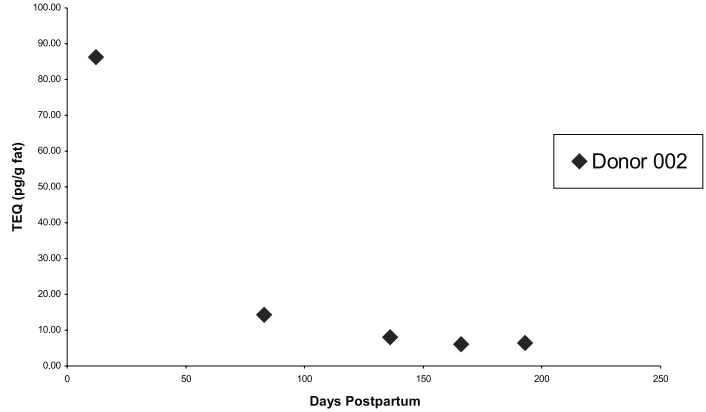

Figure 1. Dioxin/furan TEQs in breast milk (pg/g fat): decreasing 2-28 weeks after delivery.

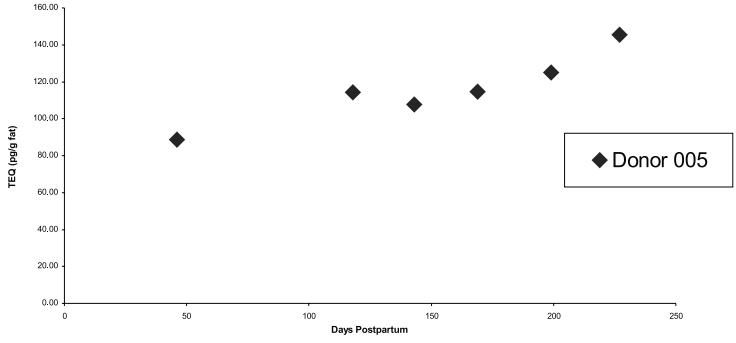

Figure 2. Dioxin/furan TEQs in breast milk (pg/g fat): increasing trend 6-32 weeks after delivery.

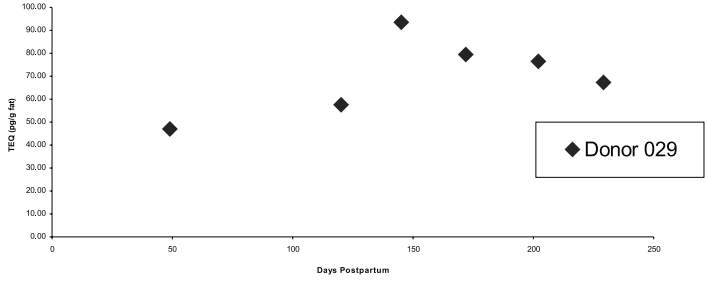

Figure 3. Dioxin/furan TEQs in breast milk (pg/g fat): unclear trend 7-32 weeks after delivery.

measure of TEQ. Comparative analyses of breast milk samples from the time-course study by CALUX and HRGC/HRMS will further describe the inter-individual variation in the kinetics of off-loading POPs chemicals in a population, and clarify the precision of the CALUX assay.

Acknowledgements

We express our appreciation to the women of Kazakhstan who volunteered for this study. This study was funded in part by Trout Farm Ltd., National Institute of Environmental Health Sciences, US Environmental Protection Agency, and a Small Business Innovation Research Grant from NIEHS ES 08372-03,.

References

1. Hooper K., Petreas M.X., She J, Visita P., Winkler J., McKinney M., Mok M., Sy F., Garcha J., Gill M., Stephens R.D., Semenova G., Sharmanov T., Chuvakova T. (1997) Environ Health Perspect. 105, 1250-1254.
2. Hooper K., Petreas M.X., She J., Visita P., Winkler J., McKinney M., Wade T.J., Stephens R.D., Chuvakova T., Kazbekova G., Druz N., Sharmanov T., Hayward D., Grassman J. (1998) Environ Health Perspect. 106, 1-10.

3. She J., Petreas M.X., Visita P., McKinney M., Sy F., Winkler J., Hooper K. (1998) Chemosphere 37, 431-442.
4. Hooper K., Chuvakova T., Kazbekova G., Hayward D., Tulenova A., Petreas M.X., Wade T.J., Benedict K., Cheng Y.Y., Grassman J. Environ. Health Perspect. (1999) 107, 447-457.
5. WHO. Levels of PCBs, PCDDs and PCDFs in Breast Milk: Results of WHO-coordinated Interlaboratory Quality Control Studies and Analytical Field Studies (Yrjanheikki EJ, ed). Environmental Health Series Report #34. Copenhagen: WHO Regional Office for Europe, 1989.
6. Patterson D.G., Isaacs S.G., Alexander L.R., Turner W.E., Hampton L., Bernert J.T., Needham L.L. (1991) p. 299-342, in Environmental Carcinogens Methods of Analysis and Exposure Assessment, vol 11:Polychlorinated Dioxins and Dibenzofurans (Rappe C, Buser HR, Dodet B, O'Neill IK, eds). IARC Scientific Publications No. 108. Lyon: International Agency for Research on Cancer.
7. Hayward D.G., Hooper K., Andrzejewski D. (1999) Anal Chem. 71, 212-220.
8. Ahlborg U.G., Becking G.C., Birnbaum L.S., Brower A., Derks H.J.G.M., Feeley M., Golor G., Handberg A., Larsen J.C., Liem A.K.D. (1994) Chemosphere, 28, 1049-1067.
9. Van den Berg, M., Birnbaum L., Bosveld A.T.C., Brunstrom B., Cook P., Feeley M., Giesy J.P., Hanberg A., Hasegawa R., Kennedy S., Kubiak T., Larsen J.C., van Leeuwen F.X.R., Liem A.K.D., Nolt C., Peterson R.E., Poellinger L., Safe S., Schrenk D., Tillin D., Tysklind M., Younes M., Waern F., Zacharewski T. (1998) Environ. Health Perspect. 106, 775-792.
10. Denison M.S., Brouwer A., Clark G.C. United States Patent # 5,854,010.
11. Chu M.D., Clark G.C., Patent application # 09/872,758.
12. Hooper K., Hayward D., Chu M., Anderson M., Farland W., Lucier G., and Clark G. (1999) Organohalogen Compounds 44:129-33.