ANALYSIS OF HUMAN BREAST MILK TO ASSESS EXPOSURE TO CHLORINATED CONTAMINANTS IN KAZAKHSTAN

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1. INTRODUCTION

The Hazardous Materials Laboratory was contracted by Wellstart International, a non-profit sponsor of international infant care and breast feeding programs, to participate in a USAID-sponsored project to analyze samples of breast milk and infant and adult foods from Kazakhstan for chlorinated contaminants. The study arose from two apparent facts: Infant mortality in Kazakhstan is high (greater than 40 per 1000 live births); and good breast feeding practices, which are known to increase infant survival and well being, were thought to be in decline. In-country health practitioners reported that breast feeding was decreasing because of women's concern that their milk was contaminated with chemical pollutants. The overall objective of the study was to determine the extent and level of selected organochlorine pesticides (OC), PCBs and PCDD/PCDFs in samples of breast milk collected from representative urban and rural populations from southern Kazakhstan.

2. METHODOLOGY

To identify likely contaminants and possible sources, and to develop an effective exposure assessment strategy, background information was collected on the ethnic, cultural, religious and social sub-groups, and their dietary habits. In addition, agricultural patterns, pesticide use, and major industrial processes helped identify representative sampling sites. The study design, including selection of sampling sites, selection of subjects, and development of a sampling protocol and exposure assessment questionnaire, was formulated after information was obtained on the population demographics and the likely dietary and environmental sources of exposure. It incorporated a sampling strategy that identified specific populations (urban, rural, highly exposed) and targeted specific geographic regions for sampling. For each geographic region and target population, the Ministry of Health identified and arranged access to suitable maternal and child health clinics where breast milk donors could be identified. At each site, a sufficient number (10-15) of breast milk donors were recruited to give the exposure assessment adequate power and sensitivity to assess contaminant levels in the sub-groups (e.g., Kazakh/Russian, fish eaters, etc.). A total of 97 breast milk samples were collected from seven sampling sites. Representative foodstuff (dairy, fish, fat) were collected from each site.

Sample collection was based on the WHO/EURO protocol[1] (primiparous women with 2-8 week old infant). Milk was manually expressed into chemically clean glass jars with Teflon-lined caps. All samples were kept frozen until analysis. Analyses for PCDD/PCDF

and congener-specific PCBs were conducted on individual samples when sufficient volume (100 mL) was available. When smaller volumes were available a compositing strategy was employed, with the following criteria (in order of decreasing importance): geographic region (clinic); ethnicity (Kazakh/Russian/Other, as a general descriptor of cultural/dietary habits); fish consumption; region of mother's residence (agricultural/industrial/urban). OC pesticides were analyzed in individual samples (10 mL).

For PCDD/PCDF, PCB and OC analyses, 100 g of milk were quickly thawed, spiked with internal and surrogate standards, and extracted with 2:1:1 ethanol:hexane:ethyl ether in the presence of sodium oxalate. For OC analysis, an aliquot of the organic phase, corresponding to 10 g of sample, was passed through GPC, concentrated, and analysed by GC-ECD. The remainder of the organic phase was loaded onto an AX-21 carbon column, eluted with 6:4 dichloromethane:hexane and collected as fraction I. Toluene elution of the carbon column in the reverse direction resulted in fraction II. Fraction II was cleaned up with silica gel and alumina columns, concentrated and injected into a HRGC-HRMS to measure PCDD/PCDFs and PCBs 77, 126, 169. Part of fraction I was used for fat determination and another part was digested with H_2SO_4. The organic layer was further cleaned up with silica gel and alumina. Eighty PCB congeners were quantitated by GC-HRMS[3].

3. RESULTS AND DISCUSSION

All fish and dairy products had very low levels of the target analytes. OC concentrations in breast milk are summarized in Table 1. β-HCH, p,p' DDE and HCB were measured in every sample, while α-HCH and p,p'-DDT were measured in the majority of samples. The levels of β-HCH were high, with even the minimum value exceeding what is considered a background level[2] (200 ng/g fat). In contrast, HCB levels were below background[2] (100 ng/g fat) in almost 75% of the samples.

Concentrations of PCDD/PCDFs in breast milk are presented in Table 2 and for major PCB congeners in Table 3. Very high levels of 2,3,7,8-TCDD were measured in a few samples collected in one agricultural region (High cluster). The remaining samples appeared unremarkable. Statistical analysis of measured concentrations and questionnaire data will be presented in an effort to identify exposure pathways.

4. ACKNOWLEDGEMENT

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5. REFERENCES

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Table 1. Summary statistics of OC concentrations (ng/g fat) in 101 human milk samples

	# > DL	MIN	MAX	MEDIAN	MEAN
% Lipid	100	1.59	7.95	4.01	4.14
β-HCH	100	430	8610	1670	2210
4,4-DDE	100	240	10540	1500	1960
HCB	100	6	440	78	91
4,4-DDT	99	75	1030	230	300
α- HCH	98	15	670	60	78
2,4-DDT	12	9	49	28	31
2,4-DDE	5	15	44	29	28
Aldrin	5	12	47	13	21
γ-HCH	4	8	490	20	135
4,4-DDD	4	13	21	18	17
Oxychlordane	2	17	66	41	41
Endosulfan-II	2	12	17	15	15
Mirex	1	10	10	10	10
trans-Nonachlor	1	4	4	4	4

Non detected: Heptachlor, heptachlor epoxide, dieldrin, endrin aldehyde, 2,4-DDD methoxychlor, chlordecone, α–chlordane, γ–chlordane

Table 2. Summary statistics of PCDD/PCDF concentrations (pg/g fat) in human milk from the High Cluster and All samples.

	HIGH CLUSTER (N=8)				ALL (N=40)			
	MIN	MAX	MEDIAN	MEAN	MIN	MAX	MEDIAN	MEAN
%Lipid	1.59	7.95	5.27	4.98	1.59	7.95	4.50	4.63
2,3,7,8-Cl4DD	6.36	118	22.7	46.5	1.26	118	5.44	13.6
1,2,3,7,8-Cl5DD	3.14	35.6	10.5	13.2	0.41	35.6	2.52	4.45
1,2,3,4,7,8-Cl6DD	1.05	4.38	1.80	2.15	0.29	4.38	0.97	1.15
1,2,3,6,7,8-Cl6DD	3.42	13.1	5.25	6.78	1.04	13.1	3.56	4.01
1,2,3,7,8,9-Cl6DD	0.73	3.14	1.41	1.65	0.28	4.65	0.88	1.14
1,2,3,4,6,7,8-Cl7DD	5.67	23.9	8.31	10.7	3.35	31.9	8.34	10.0
1,2,3,4,6,7,8,9-Cl8DD	38.1	475	105	151	38.1	475	85.8	112
2,3,7,8-Cl4DF	0.31	2.52	0.52	0.84	0.16	4.50	0.69	1.09
1,2,3,7,8-Cl5DF	0.18	1.96	0.40	0.76	0.15	3.14	0.46	0.77
2,3,4,7,8-Cl5DF	2.91	5.67	4.40	4.23	1.58	17.2	4.51	5.29
1,2,3,4,7,8-Cl6DF	1.16	2.32	1.68	1.71	0.79	6.18	1.98	2.28
1,2,3,6,7,8-Cl6DF	1.36	2.32	1.74	1.75	0.63	5.02	1.65	1.91
1,2,3,7,8,9-Cl6DF	0.35	2.52	0.43	0.75	0.16	4.07	0.63	0.75
2,3,4,6,7,8-Cl6DF	0.78	3.14	1.03	1.39	0.47	5.23	0.95	1.18
1,2,3,4,6,7,8-Cl7DF	1.55	3.87	2.06	2.41	0.80	5.75	2.11	2.40
1,2,3,4,7,8,9-Cl7DF	0.38	3.14	0.61	0.91	0.30	4.07	0.78	1.03
1,2,3,4,6,7,8,9-Cl8DF	0.88	12.6	2.63	3.74	0.88	12.6	2.75	3.00
I-TEQ	11.6	133	31.0	57.2	4.20	133	11.9	20.1

Table 3. PCB Levels in Human Milk (ng/g fat, n=38)

PCB	Min	Max	Median	Mean	Mean % of Total
28	2.8	120	10.5	18.8	5.1
Total Tri-	3.1	125	11.0	19.8	5.4
52	0.4	13.5	1.4	1.8	0.5
74	9.7	130	26.2	32.5	8.8
66	0.6	31.1	5.3	7.1	1.9
56/60	0.4	12.7	2.3	2.6	0.7
77	0.005	0.03	0.01	0.01	0.003
Total Tetra-	15.7	193	35.5	46.6	12.7
101	0.1	9.7	1.8	2.1	0.6
99	3.7	80.4	19.9	22.8	6.2
87	0.5	7.1	1.1	1.3	0.4
118	9.7	132	24.8	26.7	7.3
105	0.8	27.9	6.8	8.3	2.2
126	0.05	0.4	0.07	0.09	0.02
Total Penta-	28.5	262	61.0	65.3	17.7
149	0.3	6.4	1.1	1.8	0.5
146/161	1.3	9.1	2.2	4.5	1.2
153	20.4	276	49.6	64.9	17.6
138	10.5	205	41.5	58.9	16.0
128/162	0.3	9.3	2.0	3.0	0.8
156	0.1	23.0	5.5	15.4	4.2
169	0.02	0.07	0.03	0.03	0.008
Total Hexa-	37.9	544	109	152	41.3
187	1.6	69.9	5.4	8.8	2.4
183	0.3	41.8	2.6	4.4	1.2
177	0.1	18.2	2.0	3.0	0.8
171	0.04	5.4	1.4	1.6	0.4
180	9.1	136	24.1	30.5	8.3
170	2.7	128	7.1	10.8	2.9
Total Hepta-	17.1	252	49.3	63.9	17.3
Total Octa-	7.8	176	11.4	20.0	5.4
Total Nona-	0.2	2.2	0.6	0.7	0.2
Deca-CB	0.06	0.4	0.1	0.2	0.05
Total PCBs	149	1330	288	368	100