Analysis of Breast Milk to Assess Exposure to Chlorinated Contaminants in Kazakhstan: High Levels of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) in Agricultural Villages of Southern Kazakhstan

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To assess levels of chlorinated contaminants in breast milk, we measured organochlorine pesticides, polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) in breast milk samples collected in 1994 according to the World Health Organization protocol from 92 donors that were representative of rural population in southern Kazakhstan. High levels (10–120 pg/g fat) of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the most toxic of the PCDD/PCDF congeners, were found in breast milk samples from an agricultural region. TCDD was the major contributor (75%) to the international toxicity equivalents of these samples. The same distinctive PCDD/PCDF congener pattern was found in 15 breast milk samples and 4 serum samples collected in 1996 in a follow-up study, and has now been confirmed by three analytical laboratories. Key words: breast milk, co-planar PCBs, dioxins, exposure assessment, furans, Kazakhstan, TCDD. Environ Health Perspect 106:797–806 (1998). [Online 13 November 1998] http://ehpnet1.niehs.nih.gov/docs/1998/106p797-806hooperabstract.html

In the first comprehensive profile of lipophilic xenobiotics in a country of the former Soviet Union, a broad panel of analytes (60 organochlorine (OC) and congener-specific dioxins, furans, and polychlorinated biphenyls (PCBs)) was measured in breast milk samples from the general population of Kazakhstan (1,2). The study was undertaken because of concerns about breast milk contamination, which could lead to decreases in breast-feeding and increases in birth rate and infant mortality.

Breast milk offers a convenient and non-invasive means of assessing levels of persistent lipophilic xenobiotics in humans. Several public health and environmental benefits result from monitoring breast milk for persistent contaminants. First, such data provide insight into adult body burdens, historical human exposures, and environmental conditions. Second, these measures complement general environmental monitoring and provide a more accurate assessment of human exposures. Finally, perinatal doses to the fetus and the nursing infant can be estimated. For these reasons, many studies have measured concentrations of chlorinated contaminants in breast milk (1,3–6).

Several of the chlorinated contaminants have estrogenlike activity (7–11) and have been linked to health effects as diverse as shortened duration of lactation in mothers (12) and neurodevelopmental deficits and intellectual impairment in children (13,14). For polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs/PCDFs), contaminant levels in breast milk (15–18) and human health effects (11,15,19,20) have been recently reviewed and, for TCDD, include cancer (15,21–24) and an altered infant sex ratio (25).

Organochlorine pesticides, PCBs, PCDDs, and PCDFs were measured in samples of breast milk and a variety of infant and adult foods collected from regional populations in southern Kazakhstan over a 4-week period beginning in February 1994. Results for total PCBs and OC pesticides were presented in companion papers (1,2). The present paper discusses the levels of PCDDs/PCDFs and co-planar PCBs found in 1994, as well as the levels of PCDDs/PCDFs measured in breast milk and serum samples collected in 1996 in a follow-up study.

Materials and Methods

Study design. Rationale and background for the original study (1994), as well as study design, exposure assessment and demographic questionnaire, target analytes, World Health Organization Regional Office for Europe (WHO/EURO) protocol for breast milk sample collection (26), compounding strategy, and statistical analysis, are described in detail elsewhere (1). Primiparous breast milk donors with healthy infants 2–8 weeks of age (WHO criteria) (26) were accessed through regional maternal and child health clinics (MCHCs), as arranged by the Kazakhstan Ministry of Health. Donors (n = 7–15) were enrolled at each MCHC to assess chlorinated contaminant levels for that region. Sampling sites were selected to reflect major industrial, agricultural, and population centers of southern Kazakhstan. Donors were enrolled from rural and urban populations, as well as from targeted populations with potential for high exposure (e.g., residents near the Aral Sea, Caspian Sea, or cotton-growing regions, or with high fish consumption).

In 1994, four populations were sampled at seven MCHCs: 1) large urban centers including Almaty (capital and government center) and Shymkent and Qyzyl-Orda (large industrial cities of the south); 2) Aralk, a large fishing village on the Aral Sea (elevated fish consumption); 3) Atyna, on the Caspian Sea near the Tengiz oilfields (elevated fish consumption); and 4) two agricultural villages combined into the “rural site” (cotton-growing region). In 1996, the two villages were resampled.

Composite samples. PCDD/PCDF and congener-specific PCB analyses require a large volume (100 ml) of breast milk, which is provided by either an individual donor (100 ml

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sample) or many donors (a composite of samples, each <100 ml). The initial objective was to survey regional populations for contaminant levels. The sample design called for extensive compositing, and donors were not asked to provide 100 ml samples. During the later stages of sampling, particularly in the rural region, individual samples of 100 ml were encouraged and generally obtained. Of the 92 primiparous milk donors sampled country-wide in 1994, 17 women provided sufficient volume (100 ml) to permit analysis of PCDD/PCDFs, PCBs, and OCs (I). Milk samples of lesser volume were combined to form 23 composites of 100 ml each.

Composites were made by combining equal volumes of milk from each of the contributing donors; the number of donors per composite ranged from 2 to 8, with a mean of 2.9. Compositing criteria were (in decreasing order of importance) geographic region defined by MCHC; ethnicity (Kazak/Russian/other, which reflects cultural/dietary habits); fish consumption; and characteristic of mother’s residence (agricultural, industrial, or urban).

In November 1996, researchers returned to the rural site to collect breast milk samples (100 ml) from an additional 15 primiparae. Blood samples (20 ml) were also collected in 1996 from four mothers who had donated breast milk in 1994 to determine the time course of off-loading of TCDD by lactation. Breast milk and serum samples were analyzed for PCDDs/PCDFs.

**Blood sample collection.** Blood samples (20 ml) were collected in two 10-ml red top nonsilicone-coated vacutainers and allowed to clot for 30 min. Serum was prepared by centrifugation at 1,000g for 10 min, transfer and centrifugation in a second tube, and transfer to a glass vial, which was sealed with a Teflon-lined stopper/crimped aluminum fastener. Samples were immediately frozen and maintained at -20°C.

**Analytical methods.** Breast milk, cow’s milk, and fish samples collected in 1994 were prepared and analyzed by the Hazardous Materials Laboratory (HML) (1,2,27). Duplicate analyses were run on 10% of the human milk samples (4/40). The relative percent difference (RPD) for TCDD on those duplicates ranged from 2 to 14%, with a mean of 6%. All TCDD measurements were above the reporting limit of 1 pg/g fat.

Breast milk and serum samples from the 1996 study were analyzed by laboratories at the U.S. Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention (CDC), respectively. Breast milk samples were spiked with 15 13C12-labeled PCDD/PCDF standards and extracted using a modified Association of Official Analytical Chemists (AOAC) extraction procedure (2:1:1 ethanol:hexane:ethyl ether in the presence of sodium oxalate) (28). Lipids were determined gravimetrically (29); cleanup is described elsewhere (30). Extracts were analyzed by quadrupole ion storage mass spectrometry/mass spectrometry (MS/MS) using multiple reaction monitoring and multiple frequency irradiation (31), modified from the method used by Plomley et al. (32). TCDD results were based on the average quantitation of three daughter ions (257, 259, and 287), with relative response factors (RRFs) derived from a 4-point calibration curve (0.8–30 pg) that was verified daily. Precision and accuracy for TCDD in breast milk and food using quadrupole ion storage MS/MS is commonly >80% at a quantitation limit of 0.3–1.0 pg/g fat, depending upon the total lipid available.

Serum samples were analyzed for PCDDs/PCDFs by high-resolution gas chromatography (GC)/isotope dilution high-resolution MS based upon published methods (33,34). Analytes were separated from samples by a solid phase extraction procedure followed by a multicolumn automated cleanup procedure. The analytes were separated on a DB-5 or SP2331 capillary column and quantified using high-resolution (10,000 resolving power) MS capable of selected ion monitoring. Individual analyte concentrations were determined by comparing RRFs generated using isotopically labeled and known native standard concentrations.

Residue levels are expressed as picograms per gram milk lipid. For all reported data, international toxicity equivalents (I-TEQs) for PCDDs/PCDFs and PCBs are based upon the WHO-TEQ system (35).

**Statistical analyses.** Only measurements above the detection level were used in the statistical analyses and are reported in the tables. Analytical data were stored in EXCEL 5.0 (Microsoft, Redmond, WA) and ACCESS 97 (Microsoft, Redmond, WA), and questionnaire data were stored in ACCESS 97. All statistical analyses were conducted in STATA 5.0 (Stata Corp, College Station, TX). Distributions of each chemical compound were examined for symmetry, multimodality, and normality. All congener concentrations in breast milk were found to be skewed by a test for normality.

**Table 1. Summary statistics for demographic variables for rural and nonrural populations sampled in 1994 and 1996**

| Variable                        | 1994/1996 | 1994 | 1996 |
|--------------------------------|-----------|------|------|
| **Ethnicity of donors**         |           |      |      |
| Kazak                          | 70 (74)   | 13 (100) | 14 (93.3) |
| Russian                        | 24 (25)   | 0 (0) | 0 (0) |
| Uzbek                          | 1 (1)     | 0 (0) | 0 (0) |
| Total                          | 95 (100)  | 13 (100) | 15 (100) |
| **Infant’s sex**               |           |      |      |
| Male                           | 58 (61)   | 10 (77) | 8 (53) |
| Female                         | 37 (39)   | 3 (23) | 4 (2%) |
| Total                          | 95 (100)  | 13 (100) | 12 (100) |
| **Mean maternal age (years)**  | 22.6 ± 3.91 | 21.54 ± 3.89 | 21.33 ± 2.29 |
| **Mean mother’s BMI ± SD**     | n = 95    | n = 13 | n = 9 |
| **Mean infant age (day)**      | 40.87 ± 15.88 | 34.92 ± 13.16 | 34.78 ± 14.8 |
| **Mean infant birth weight (g)** | 3,294 ± 503 | 3,500 ± 479 | 3,383 ± 356 |

Abbreviations: SD, standard deviation; BMI, body mass index.

*Data missing.

*Number of donors from each site (percent).
Natural log transformation was used when making comparisons for highly skewed distributions. One-way analysis of variance (ANOVA) was used to compare mean concentrations of PCDDs/PCDFs for the different sampling areas. One-way ANOVA tests were followed by Bonferroni-adjusted multiple pairwise comparisons. The t-test was used to compare levels in rural versus non-rural regions. All p-values are two-sided, and p-values ≤0.05 were considered significant.

Results

Demographic Characteristics

Demographic characteristics for the donor populations from the six sampling sites of 1994 and the rural site of 1996, as well as the combined 1994–1996 donors, are shown in Table 1. The rural primiparae sampled in 1994 and 1996 have similar demographic characteristics. Russians were more prevalent among donor populations sampled from nonrural sites than from the rural site (Fisher’s exact test, p = 0.001), consistent with the distribution of ethnicities in Kazakhstan. Russians are more prevalent in the northern cities of Almaty and Atyrau than in the southern cities of Aralsk, Qyzyl-Orda, and Shymkent and the rural region (Table 1). There were no significant demographic differences [maternal age or body mass index (BMI), infant age, birth weight, or sex] between breast milk donors from the five nonrural sites of 1994 and the rural site of 1994 and 1996. Mothers sampled in 1996 were marginally older than those sampled from the rural region in 1994 (23.3 vs. 20.75 years).

Analytical Results

PCDDs/PCDFs. In the 1994 study, the 17 2,3,7,8-substituted PCDDs/PCDFs were measured in 40 breast milk samples (17 individuals and 23 composites). The range and mean values of individual and composite samples were similar by region and ethnic composition, and results were combined in the analyses. Mean concentrations of the PCDD/PCDF congeners, their toxic equivalency factors (TEFs) and I-TEQs, and

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Table 2. Mean ± standard deviation (SD) levels (pg/g fat) of PCDDs and PCDFs in breast milk by location (1994)

| Congener     | Rural Mean ± SD | Almaty Mean ± SD | Atyrau Mean ± SD | Shymkent Mean ± SD | Qyzyl-Orda Mean ± SD |
|--------------|-----------------|------------------|------------------|---------------------|----------------------|
| TCDD         | 1               | 46.48* ± 45.38   | 6.62* ± 5.13     | 3.36** ± 1.65      | 6.66** ± 2.59        |
| PentacDD     | 0.5             | 14.60* ± 10.93   | 2.39* ± 1.23     | 1.91** ± 1.08      | 3.97** ± 1.10        |
| 1,2,3,4,7,8-HexacDD | 0.1         | 2.10 ± 1.16      | 0.87             | 0.8                 | 1.82                |
| 1,2,3,6,7,8-HexacDD | 0.1       | 7.84* ± 3.85     | 4.80 ± 1.81      | 2.56** ± 0.90      | 3.73 ± 0.67         |
| 1,2,3,7,8,9-HexacDD | 0.1       | 1.51 ± 0.66      | 0.87             | 0.54 ± 0.08        | –                   |
| 1,2,3,4,6,7,8-HeptacDD | 0.01     | 11.41 ± 6.13     | 8.72 ± 1.28      | 7.66 ± 4.51        | 9.62 ± 2.67         |
| OctacDD      | 0.001           | 168.95 ± 141.06  | 83.67 ± 25.60    | 105.04 ± 61.51     | 88.80 ± 70.23       |
| TetraCDF     | 0.1             | –                | 0.93 ± 0.30      | 0.61 ± 0.26        | 2.81 ± 1.49         |
| 1,2,3,7,8-PentaCDF | 0.05       | 0.18             | –                | 1.28 ± 0.45        | 2.20 ± 0.22         |
| 2,3,4,7,8-PentaCDF | 0.5        | 4.23** ± 0.93    | 5.35 ± 1.32      | 4.25** ± 2.04      | 12.06* ± 4.53       |
| 1,2,3,4,7,8-HexacDD | 0.1      | 1.71** ± 0.39    | 2.23 ± 0.43      | 1.88** ± 0.85      | 4.09 ± 1.24         |
| 1,2,3,6,7,8-HexacDD | 0.1       | 1.76 ± 0.36      | 1.71 ± 0.53      | 1.55** ± 0.57      | 3.48** ± 1.20       |
| 1,2,3,7,8,9-HexacDD | 0.1       | 0.35             | –                | 0.48               | –                   |
| 2,3,4,6,7,8-HexacDD | 0.1      | 1.21 ± 0.4       | 0.84** ± 0.04    | 0.69** ± 0.24      | 1.88 ± 0.24         |
| 1,2,3,4,6,7,8-HeptacDD | 0.01     | 2.34 ± 0.91      | 2.34 ± 0.58      | 1.40** ± 0.67      | 3.03* ± 0.58        |
| 0.01         | –              | –                | –                | –                   | –                   |
| OctaCDF      | 0.001           | 3.74 ± 3.70      | 3.26 ± 0.56      | 2.81 ± 1.69        | 3.81               |
| I-TEQ        | NA              | 57.22* ± 47.98   | 11.89* ± 6.30    | 7.45* ± 3.33       | 16.95* ± 5.69       |
| PentacDD/TCDD | NA            | 0.42 ± 0.22      | 0.40 ± 0.08      | 0.67 ± 0.27        | 0.62 ± 0.08         |

Abbreviations: PCDDs, polychlorinated dibenzo-p-dioxins; PCDFs, polychlorinated dibenzofurans; TEF, toxic equivalency factor; ND, not done (small sample size); NS, no significant pairwise comparisons; I-TEQ, international toxicity equivalent. One-way ANOVA tests were followed by Bonferroni-adjusted multiple pair-wise comparisons.

**Indicates sites that had significantly different levels from sites marked by one asterisk at the cited level of significance.
Tables of chlorinated contaminants in breast milk. (A) TCDD and international toxicity equivalent (I-TEQ). (B) Dioxin-like polychlorinated biphenyls (PCBs). (C) Chlorinated pesticides. \( \beta \)-HCH, \( \beta \)-hexachlorocyclohexane. Error bars indicate standard deviation.

**Table 3. Mean ± standard deviation (SD) levels (pg/g fat) of PCDDs/PCDFs in urban and rural areas (1994 and 1996)**

| Congener               | Rural areas (1996) | Rural areas (1996/1994) | Nonrural areas (1996) | p-Value* |
|------------------------|--------------------|-------------------------|------------------------|----------|
|                        | No. | Mean ± SD | No. | Mean ± SD | No. | Mean ± SD |               |
| TCDD                   | 15  | 28.43 ± 27.64 | 15  | 34.71 ± 34.88 | 32  | 5.34 ± 3.21 | <0.0001      |
| PentaCDD               | 15  | 12.4 ± 8.73  | 15  | 13.10 ± 9.28  | 27  | 2.46 ± 1.31  | <0.0001      |
| 1,2,3,4,7,8-HexaCDD    | 15  | 4.53 ± 2.03  | 15  | 3.75 ± 2.11  | 4   | 1.06 ± 0.51  | 0.0022       |
| 1,2,3,6,7,8-HexaCDD    | 15  | 6.29 ± 3.21  | 21  | 6.67 ± 3.36  | 30  | 3.35 ± 1.56  | 0.0001       |
| 1,2,3,7,8,9-HexaCDD    | 13  | 1.86 ± 1.09  | 17  | 1.78 ± 0.99  | 8   | 0.68 ± 0.52  | 0.008        |
| 1,2,3,4,6,7,8-HeptaCDD | 15  | 11.33 ± 5.88 | 22  | 11.35 ± 5.18 | 31  | 9.95 ± 5.57  | 0.38         |
| OctaCDD                | 15  | 137.07 ± 108.89 | 22  | 114.57 ± 117.44 | 32  | 101.80 ± 52.07 | 0.062 |
| TetraCDF               | 15  | 0.58 ± 0.27  | 15  | 0.58 ± 0.27  | 19  | 1.37 ± 1.36  | 0.049        |
| 1,2,3,7,8-PentaCDF     | 15  | 0.77 ± 0.46  | 16  | 0.73 ± 0.47  | 5   | 1.61 ± 0.83  | 0.007        |
| 2,3,4,7,8-PentaCDF     | 15  | 5.77 ± 2.46  | 23  | 5.24 ± 2.16  | 31  | 5.65 ± 4.01  | 0.054        |
| 1,2,3,4,7,8-HexaCDF    | 15  | 2.82 ± 0.99  | 23  | 2.43 ± 0.99  | 31  | 2.41 ± 1.30  | 0.94         |
| 1,2,3,6,7,8-HexaCDF    | 15  | 3.19 ± 1.47  | 23  | 2.78 ± 1.41  | 31  | 1.90 ± 1.07  | 0.014        |
| 1,2,3,7,8,9-HexaCDF    | 0   | NA           | 1   | 0.35         | 1   | 0.48        | ND           |
| 2,3,4,6,7,8-HexaCDF    | 13  | 2.12 ± 0.92  | 18  | 1.87 ± 0.90  | 16  | 1.07 ± 0.54  | 0.0001       |
| 1,2,3,4,6,7,8-HeptaCDF | 15  | 5.19 ± 2.23  | 21  | 4.38 ± 2.33  | 28  | 2.34 ± 1.24  | 0.0003       |
| 1,2,3,4,7,8,9-HeptaCDF | 4   | 1.58 ± 0.97  | 4   | 1.58 ± 0.97  | 0   | ND          | ND           |
| OctaCDF                | 15  | 15.7 ± 18.01 | 23  | 11.5 ± 15.65 | 26  | 2.89 ± 1.31  | 0.0072       |
| I-TEQ                  | 15  | 39.93 ± 29.82 | 23  | 45.95 ± 37.00 | 32  | 10.80 ± 5.68  | <0.0001      |
| PentaCDD/TCDD          | 15  | 0.56 ± 0.34  | 22  | 0.52 ± 0.31  | 27  | 0.51 ± 0.18  | 0.90         |

Abbreviations: PCDD, polychlorinated dibenzo-p-dioxin; PCDF, polychlorinated dibenzofuran; I-TEQ, international toxic equivalent.

* Determined by t-test for mean PCDD/PCDF difference between rural and nonrural areas (1994/1996).

With the combined data set, mean TCDD, pentaCDD, and I-TEQ levels are again strikingly higher in breast milk samples from the rural site (1994) and in milk and sera collected in 1996 (Fig. 3). The pentaCDD/TCDD ratios do not differ significantly at different sampling sites (Table 2).

TCDD, pentaCDD, heptaCDD, and octaCDD levels in all samples collected in 1994 and 1996 are presented in Figure 2. While levels of TCDD and pentaCDD in the rural area are quite different from levels in nonrural areas, the levels of heptaCDD and octaCDD are similar in the rural and nonrural areas and appear to be background contamination.

**PCDD/PCDF correlations.** Correlations between PCDD/PCDF congeners differ for the rural and nonrural regions. In nonrural...
areas, TCDD levels correlated with the levels of other PCDDs/PCDFs: they were highly correlated with pentaCDD and 1,2,3,6,7,8-hexaCDD ($r = 0.82$ and 0.69, respectively) and mildly correlated with 1,2,3,6,7,8-hexaCDF ($r = 0.58$). In women with low TCDD levels (<20 pg/g), the TCDD–pentaCDD correlation was strong ($0.79$). In rural areas TCDD was not correlated with the other PCDDs, and the non-TCDD PCDDs were strongly correlated: pentaCDD with 1,2,3,6,7,8-hexaCDD ($r = 0.77$) and heptaCDD with octaCDD ($r = 0.82$). The PCDFs were also highly correlated: tetraCDF and 1,2,3,7,8-pentaCDF ($r = 0.82$), 1,2,3,4,7,8-hexaCDF and 1,2,3,6,7,8-hexaCDF ($r = 0.81$), and 1,2,3,7,8-pentaCDF and 2,3,4,6,7,8-hexaCDF ($r = 0.87$). In women with high TCDD levels (>20 pg/g; all in the rural area), few correlations were found: 1,2,3,4,7,8-heptaCDF with octaCDF ($r = 0.87$); TCDD was only slightly correlated with pentaCDD ($r = 0.22$).

**Demographic correlations.** Although no significant correlation was found between I-TEQ and maternal age, or between any dioxin or furan and infant age, several PCDD/PCDF congeners were significantly correlated with demographic variables.

**Mother.** Three congeners (pentaCDD, tetraCDF, and 1,2,3,6,7,8-hexaCDF) were positively correlated with mother’s BMI ($r = 0.38$, 0.47, and 0.43, respectively). One PCDD and five PCDFs (1,2,3,4,7,8-hexaCDD, tetraCDF, both pentaCDFs, and 1,2,3,6,7,8- and 2,3,4,6,7,8-hexaCDF) positively correlated with mother’s age ($r = 0.47$; correlation coefficients between 0.50 and 0.53). Whether any of these correlations have biological significance is not clear because the effects of possibly confounding variables are not assessed by these pairwise correlations. The lipid content of breast milk varied (1.9–8.3%), but no relationship was found between percent of lipid content and mother’s BMI or levels of TCDD, pentaCDD, or I-TEQ.

**Infant.** Sample size limited the power to detect correlations between TCDD levels and infant demographic variables. For example, at our sample size ($n = 23$ individuals, or 23 individuals + 72 composites), a mean difference in infant birth weights of at least 400–500 g is required for detection with 80% statistical power, i.e., required for infant birth weights to be considered significantly different (power = 0.8; $\alpha = 0.05$). Likewise, a 40–50% difference in sex ratio is required for significance.

Birth weights were reported by the mothers. Current weights of infants were either measured or, where it was too cold to weigh infants, were estimated by the physicians. No significant relationships were seen between TCDD levels and birth weight or current weight of infants, although birth weights and TCDD levels were slightly positively correlated. Mean birth weights were roughly the same (3,393 v. 3,301 g; $p = 0.41$) for infants from primiparous with either high (>20 pg/g; $n = 14$) or low (<20 pg/g; $n = 16$) TCDD levels.

Sex ratios of infants from two groups of primiparous (high or low TCDD levels) were not significantly different. Sex ratios of infants from individual primiparous with high (>20 pg/g; $n = 14$) and low (<20 pg/g; $n = 18$) TCDD levels were roughly the same (50% vs. 44% female). Sex ratios of infants from all primiparous (individual + composite samples) with high (>20 pg/g; $n = 16$) and low (<20 pg/g; $n = 79$) TCDD levels were roughly the same (44% vs. 38% female).

Six primiparous with the highest TCDD levels (all in the rural region: 30, 31, 46, 62, 116, and 118 pg/g) had a marked disparity in sex ratio: five of their infants were male and one was female. This contrasts with sex ratio of infants of all primiparous enrolled from the rural region ($n = 27$; includes composites): 16 infants were male and 11 were female.

**Dietary correlations.** Alcohol consumption and cigarette smoking were rare among study participants. TCDD levels in breast milk were contrasted with food consumption (self-reported dietary intake of selected foods): evaluation of food-frequency questionnaire data for 32 individual breast milk donors and 2 members of a composite (food frequencies were averaged) suggested relationships between intake frequencies of certain foods and TCDD levels. Considering the entire 1994/1996 donor population, mean TCDD levels were higher among women whose diet included cottonseed oil [31 pg/g (ever eaten) vs. 13 pg/g (never eaten); $p < 0.02$] or kefir, fermented cow’s milk [37 pg/g (ever) vs. 13 pg/g (never); $p < 0.02$]. When considering only women from the rural site, the relationship between TCDD level and cottonseed oil consumption did not hold: TCDD levels were not significantly higher among women who consumed cottonseed oil (37 vs. 26 pg/g). However, since most women in the rural area ate cottonseed oil (18/23), the power to examine this relationship was limited. In contrast, the relationship between TCDD levels and kefir persisted in the rural area: TCDD levels were higher among those whose diet included kefir (47 vs. 19 pg/g).

No TCDD has been found in samples of cottonseed oil analyzed thus far (quantitation limit: 0.2 pg/g) (I). Surface soil samples ($n = 2$) collected at cotton storage bins showed no unusual chemical profiles (I).

**TCDD off-loading by lactation.** Serum samples were collected in 1996 from four mothers (A–D) who were breast milk donors in 1994. Levels of the 17 2,3,7,8-substituted PCDDs/PCDFs in the 1994 breast milk and 1996 serum samples collected from these donors are shown in Table 4, and TCDD and non-TCDD I-TEQ (I-TEQ minus TCDD) values are shown in Figure 3. The nondetects (NDs) found in the serum samples in 1996 (Table 4) are consistent with levels measured in breast milk samples in 1994. Detection limits (picograms TCDD per gram lipid) are much higher in serum than breast milk due to the smaller volume and lower lipid content (3–8% vs. 4%) of serum. Thus, mean OctaCDD levels in 1994 breast milk and 1996 serum samples appear different in Table 4 (roughly 100 pg/g fat vs. nondetected levels), but are consistent because the detection limit in serum for octaCDD was 600–800 pg/g fat.

Two mothers (B and C) gave individual (100 ml) milk samples. A third mother (D) was part of a two-person composite, and the fourth (A) was part of a four-person composite. PCDD/PCDF levels in breast milk from B, C, and D (1994) are compared with levels in serum samples (1996) in Table 4.

**Off-loading of TCDD by lactation**. Serum samples were collected in 1996 from four mothers (A–D) who were breast milk donors in 1994. Levels of the 17 2,3,7,8-substituted PCDDs/PCDFs in the 1994 breast milk and 1996 serum samples collected from these donors are shown in Table 4, and TCDD and non-TCDD I-TEQ (I-TEQ minus TCDD) values are shown in Figure 3. The nondetects (NDs) found in the serum samples in 1996 (Table 4) are consistent with levels measured in breast milk samples in 1994. Detection limits (picograms TCDD per gram lipid) are much higher in serum than breast milk due to the smaller volume and lower lipid content (3–8% vs. 4%) of serum. Thus, mean octaCDD levels in 1994 breast milk and 1996 serum samples appear different in Table 4 (roughly 100 pg/g fat vs. nondetected levels), but are consistent because the detection limit in serum for octaCDD was 600–800 pg/g fat.

Two mothers (B and C) gave individual (100 ml) milk samples. A third mother (D) was part of a two-person composite, and the fourth (A) was part of a four-person composite. PCDD/PCDF levels in breast milk from B, C, and D (1994) are compared with levels in serum samples (1996) in Table 4.

**Dioxinlike PCBs.** In the 1994 study (I), PCBs were measured in 40 breast milk samples. Results for total PCBs, sum of the six prevalent “marker” PCBs, and PCB-TEQ values calculated using the three co-planar PCBs and PCBs 105, 118, 123, 156, 170, and 180 are reported elsewhere (1,2). Figure 1B gives results for the dioxinlike PCBs (mono- or di-ortho) expressed as PCB-TEQs (35) for the six samples region selected in 1994. The mean PCB-TEQ value for the six southern Kazakhstan sites was 22 pg/g fat, similar to PCB-TEQs of industrialized western European countries (I). Atyrau had the highest levels of dioxinlike PCBs (PCB-TEQ = 46 pg/g fat), mostly due to the mono-ortho congeners (I).

Correlations were found between PCB congeners and the higher chlorinated (non-TCDD) PCDDs and PCDFs in southern Kazakhstan.
Kazakhstan, suggesting that these are background exposures. From the 1994 data, pentaCDD, 1,2,3,4,7,8-hexaCDD, and 1,2,3,4,6,7,8-heptaCDD correlate with PCB 126 (r = 0.71, p<0.0001; r = 0.77, p = 0.005; and r = 0.53, p = 0.05; respectively). 1,2,3,4,7,8-HexaCDD and 1,2,3,4,6,7,8-hepta-CDD correlate with PCB-TEQ (r = 0.77, p<0.0009; r = 0.55; p<0.05; respectively). Several of the PCDFs (tetraCDF, 2,3,4,7,8-pentaCDF, 1,2,3,4,7,8-hexaCDF, 1,2,3,6,7,8-hexaCDF) correlate with PCB 169 (r = 0.50–0.53), consistent with the fact that the highest mean levels of PCBs and PCDFs are found in milk samples from Atyrau (Table 2, Fig. 1B).

Discussion

PCDDs/PCDFs

The TCDD levels measured in breast milk samples from the rural site are among the highest reported (116–118 pg/g fat) (15). Most of the rural samples (17/23) had TCDD levels greater than 20 pg/g fat. A summary of samples from 33 countries gave a mean TCDD level of 3.4 pg/g fat, with 5th and 95th percentiles of 1–7.7 pg/g fat (15). The ratios of pentaCDD/TCDD are much lower (0.4–0.7; Table 2) than the ratios (2–3) typical of serum or breast milk from industrialized countries (15).

TCDD contributes 70–85% of the I-TEQ, in marked contrast to breast milk samples from other countries where it constitutes 5–15% of the I-TEQ (Fig. 4). The congener pattern resembles that seen in Agent Orange, a defoliant mixture of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4-dichlorophenoxyacetic acid.

PCDD/PCDF correlations. In both the rural and nonrural regions, the non-TCDD PCDDs/PCDFs correlate with each other, suggesting that they are part of the background contamination. In the nonrural region, TCDD correlates with the other PCDDs/PCDFs, suggesting that it too is background. In the rural region, TCDD does not correlate with PCDDs/PCDFs. These results suggest that TCDD is the major and distinctive contaminant in the rural region.

Demographic correlations. PCDD/PCDF levels were reported to decrease 15% over the first 6 weeks of lactation in a study of 112 breast milk donors in Germany with homogeneous background exposures (16,36). In our much smaller study groups [n = 32 total individuals; rural (n = 21) and nonrural (n = 11) populations], we saw no significant decrease in TCDD with increasing length of lactation period (weeks 2–8 of infant age). Our power to detect small decreases in a small group was limited by the precision of lab measurements of TCDD (commonly 80–85%). Also, it is not clear that our study populations have similar exposure histories: e.g., Kazak women with high levels (118 pg/g) may have experienced chronic exposures; others, with low levels (6 pg/g), may no longer be exposed.

PCDDs/PCDFs and dioxinlike PCBs. Fish are likely the source of PCBs in breast milk samples from Atyrau. The Ural River drains the industrialized regions of Central Russia and bisects Atyrau as it joins the Caspian Sea at the Tengiz oilfields. Vobla fish from the Ural River, a dietary staple in Atyrau, have the highest PCB levels found in fish from Kazakhstan. It is not known whether fish are the source of the PCDF contaminants measured in breast milk samples in Atyrau.

Sources of TCDD Exposure

The highest TCDD levels (116–118 pg/g fat) in breast milk samples from the rural area approach those found in serum samples taken from occupational settings or chemical catastrophes in industrialized countries (15). However, no industrial point sources or historical accidents have
been identified as probable sources of TCDD in the rural region.

**PCBs.** PCBs are not the source of the TCDD exposures. Sites with high TCDD levels have low PCB levels, and sites with high PCB levels have low TCDD levels.  

**β-HCH.** β-Hexachlorocyclohexane (β-HCH) is used in Kazakhstan as an insecticide dip for sheep. The mean levels of β-HCH in 1994 at the six sampling sites (1,570–3,470 ng/g fat) (J) were much higher than background levels in Europe (200 ng/g fat). The highest levels were found in samples from the rural site (Fig. 1C), the location of high TCDD levels. However, β-HCH levels did not correlate with 1-TEQ, TCDD, or pentachlorodiphenyl ether (PCDE) levels in the limited number (n = 20) of 1994 breast milk samples analyzed for these compounds. β-HCH is an unlikely source of TCDD contaminants.

**Diet.** Initially, cottonseed oil seemed to be a likely source of TCDD exposure. Consumption is high in the rural region where TCDD levels are high and low in the nonrural regions where TCDD levels are low. But cottonseed oil consumption country-wide correlates with socioeconomic status, geographic location, and ethnicity. Cottonseed oil consumption is high in the cotton-growing region where the residents are predominantly Kazak and poor. Consumption is lower in the urban areas, which contain proportionally more Russians and are more wealthy. In the rural region, cottonseed oil consumption does not correlate with TCDD levels; all residents in the region consume it daily, but TCDD levels vary widely between neighbors. For the past 30 years, cottonseed oil was produced at a single state facility in Shymkent. If the oil is the source of TCDD contamination, TCDD levels should be elevated in the Kazak residents of Shymkent and Qyzl-Orda, who consume oil from this facility. However, TCDD levels in breast milk samples from these locations were not elevated.

TCDD levels did correlate with kefir consumption, both nationally and in the rural region. Unfortunately, no kefir samples were available to analyze. Whether kefir is a source of exposure, or is a confounder, is not clear.

TCDD levels did not correlate with intake frequencies of a number of foodstuffs typical of Russian or Kazak diets. These included lamb, beef, pork, chicken, eggs, fish, horse, butter, various fats, sour cream, cheese, mare’s milk, or hard white cheese. Two lamb fat samples had TCDD levels of roughly 0.5 pg/g fat (30).

Fish have classically been major sources of the biaccumulating lipophilic organochlorine compounds such as PCDDs/PCDFs, PCBs, and OC pesticides. Breast milk donors from the rural area reported low fish consumption on their food frequency questionnaire. However, fish caught from nearby Lake Chardana are a dietary staple in the summer. Fish may yet prove to be a major source of exposure to TCDD and β-HCH.

**Residence on state farms.** Chemicals used in cotton agriculture are a likely source of TCDD exposure. Defoliant is applied 2 weeks before cotton harvest. TCDD-contaminated 2,4,5-T may have been used as a defoliant. The Russians produced TCDD-contaminated 2,4,5-T during 1965–1967 at a plant in Ufa, Russia. 128 of the 250 production workers were diagnosed with chloracne, and all workers analyzed had elevated serum TCDD levels (n = 14; mean TCDD = 299 pg/g fat) (37). TCDD-contaminated stocks of 2,4,5-T or 2,4,5-trichlorophenol may have been used in agriculture in the rural region in Kazakhstan.

Years of residence on state farms may be a risk factor for TCDD exposure and could explain the different mean TCDD levels measured in 1994 and 1996. Mean TCDD levels in the rural area in 1996 (30 pg/g; n = 15) are lower than those measured in 1994 (46 pg/g; n = 8; p = 0.34). This could suggest that TCDD levels have decreased over the 2 years. However, unpublished data indicate that TCDD levels are higher in rural donors that have lived most of their lives on state farms as opposed to villages. A more likely explanation is that the rural populations sampled in 1994 and 1996 were different, with the donors sampled in 1994 having more resident-years on state farms than the donors sampled in 1996.

**TCDD off-loading by lactation.** The TCDD levels in milk (1994) and serum (1996) for women C and D are compared in Figure 3. Through 1996, these women had nursed infants for 24 and 12 months, respectively. TCDD levels in breast milk and serum were reported to be similar when age-matched groups of German women were compared (17). If we assume that TCDD levels in milk and serum are similar, the rate of TCDD off-loading from the two women over the 2-year period is roughly 4-5 pg/g lipid per month of breast-feeding.

**Effects of TCDD**

**Infant sex ratio.** Five of the six infants of women with the highest TCDD levels (>30 pg/g fat) were male. In contrast, more female infants were seen among offspring of Sevoso parents at higher TCDD levels than we observed here (25). No male and 12 female infants were born up to 7 years after the 1976 explosion to nine sets of parents with high TCDD levels in serum samples collected in 1976 (fathers: 104–2,340 pg/g fat; mothers: 238–1,650 pg/g fat). After 1984, the change in sex ratio was not significant.

A higher proportion of male than female infants might be expected in the donor population from the rural region. Primiparae with infertile sons have a stronger incentive to volunteer than mothers of new daughters because sons are more valued in the region. Primiparae may also believe that new sons are harder than new daughters and are more able to venture outside into the extreme cold of the winter sampling period. In the 1994 study, donors with male and female infants volunteered in equal numbers (6:6) in the region. In the 1996 study, 10 of the infants were male and 5 were female, so some selection bias may have occurred. Further study is required to determine whether TCDD at levels >30 pg/g affects the sex ratio in infants.

**Exposures to the nursing infant.** Exposures to the nursing infant can be estimated using the WHO-suggested nursing scenario of 6 months duration (26), 0.7 l/day ingestion rate (26), and a milk fat content of 3.5% (38). Using the highest and mean levels (118 and 35 pg/g fat) of TCDD for combined 1994–1996 data from the rural area and assuming a 5-kg infant body weight (bw), the TCDD intake levels are 578 and 172 pg/kg bw/day, respectively. Furthermore, using the highest and mean levels (132 and 46 pg/g fat) of 1-TEQ for the rural area and assuming a 5-kg infant bw, the 1-TEQ intake levels are 647 and 225 pg/kg bw/day, respectively. These are much higher than the tolerable daily intake (TDI) levels of 10 pg I-TEQ/kg bw/day in Canada and the estimated worldwide daily intake of 1–10 I-TEQ/kg bw/day (15).

The mean 1-TEQ level of breast milk samples from donors in this region (PCDDs/PCDFs and PCBs, ~80 pg/g), however, is only twice the mean 1-TEQ in breast milk samples from the United States and European countries (40–50 pg/g). This is because of the higher levels of non-PCDD PCDDs/PCDFs and planar PCBs found in samples from the United States and Europe (11,16).

It is known from studies of breast-fed and formula-fed infants of 1–5 months of age that the tetra- to hexa- congeners in breast milk are >90% absorbed by the breast-fed infant (39). Studies of autopsy tissues of five premature infants and a 3-month-old nonbreast-fed infant indicate that significant placental transport of dioxins and furans occurs. In the infant at birth,
Figure 3. Contribution of TCDD versus non-TCDD congeners to international toxicity equivalent (I-TEQ) in breast milk and serum samples from the rural area.

Table 4. Levels (pg/g fat) of PCDDs/PCDFs in breast milk and serum samples from the rural area (1994/1996)

| Congener                  | Woman and year (sample) | A* | B  | C  | D  | E  | F  |
|---------------------------|--------------------------|----|----|----|----|----|----|
|                          | 1996 (S)                 | 1996 (M) | 1996 (S) | 1996 (M) | 1996 (M) | 1996 (S) |
| TCDD                      | 6.9                      | 6.4 | 7.0 | 118.2 | 15.2 | 117.1 | 68.6 |
| PentaCDD                  | 4.7                      | 3.2 | 2.6 | 12.2  | 15.2 | 22.4  | 15.8 |
| 1,2,3,4,7,8-HexaCDD       | NR                       | 1.7 | NR | 1.4  | NR | 2.9  | NR |
| 1,2,3,6,7,8-HexaCDD       | NR                       | 3.4 | NR | 7.4  | NR | 11.5 | NR |
| 1,2,3,7,8,9-HexaCDD       | ND                       | 0.7 | ND | 0.8  | ND | 1.8  | ND |
| 1,2,3,4,7,8-HeptaCDD      | ND                       | 8.3 | ND | 6.6  | ND | 8.3  | ND |
| OctaCDD                   | ND                       | 120.1 | ND | 90.7  | ND | 88.8 | ND |
| TetraCDF                  | 2.6                      | 0.49 | 2.2 | 0.4  | 2.3 | 0.5  | ND |
| 1,2,3,7,8-PentaCDF        | ND                       | 0.49 | ND | 0.2  | ND | 0.3  | ND |
| 2,3,4,7,8-PentaCDF        | 3.3                      | 4.4 | 3.8 | 2.9  | 2.2 | 4.4  | 4 |
| 1,2,3,4,7,8-HexaCDF       | 2.6                      | 2.0 | 2.6 | 1.2  | 2.2 | 2.0  | 3.3 |
| 1,2,3,6,7,8-HexaCDF       | 2.2                      | 1.7 | 2.1 | 1.4  | 2.2 | 1.8  | 3 |
| 1,2,3,4,6,7,8-HexaCDF     | ND                       | 0.7 | ND | 0.4  | ND | 0.4  | ND |
| 2,3,4,6,7,8,HexaCDF       | ND                       | 1.0 | ND | 0.8  | ND | 1.0  | ND |
| 1,2,3,4,6,7,8-HeptaCDF    | ND                       | 1.7 | ND | 1.6  | ND | 2.0  | ND |
| 1,2,3,4,7,8,9-HeptaCDF    | ND                       | 0.7 | ND | 0.6  | ND | 0.4  | ND |
| OctaCDF                   | 4.5                      | 2.9 | 5.6 | 2.3  | 7.1 | 2.1  | 8.2 |
| I-TEQ                     | 13.31                    | 11.6 | 12.7 | 127.3 | 18.1 | 132.9 | 81.4 |
| PentaCDD/TCDD             | 0.68                     | 0.50 | 0.37 | 0.10 | 0.19 | 0.2  | 0.23 |

Abbreviations: PCDDs, polychlorinated dibenzo-p-dioxins; PCDFs, polychlorinated dibenzofurans; S, serum; M, milk; NR, not reported; ND, not detected (less than detection limit; octaCDD detection limit = 600-800 pg/g fat).

*Breast milk sample was composited with three other samples in 1994.
*Composited with one other milk sample in 1994.

the levels of the biologically active TCDD, pentaCDD, 1,2,3,6,7,8-hexaCDD, and 1,2,3,7,8-pentaCDF congeners are 25% of the levels measured in the mother’s milk (40).

It is not clear what effects, if any, these TCDD levels will have on the women, infants, and children in the rural region. The major challenges to public health in this region are infectious disease, proper nutrition, clean drinking water, and sanitation. Breast-feeding has significant benefits to the infant (nutritional, disease resistance, psychosocial, and emotional), to the community (lower infant morbidity and mortality, lower birth rate, economic), and to the mother and family. It remains the right choice in contending with the major health problems of the region until such time as there is clear evidence that the health risks presented by any contaminants simply outweigh the considerable benefits of breast-feeding.

Conclusions

This is one of the few studies that have analyzed breast milk for a broad panel of chemicals, in a systematic way, following the widely accepted WHO protocol. This is also the first thorough investigation of chlorinated contaminants in Kazakhstan. Representative samples collected from a multiethnic population over a large area provide insights into body burdens at each of the sampling locales. The major findings are as follows:

- The mean TCDD level in breast milk samples from the rural area (35 pg/g fat; range 6–118 pg/g fat) is much higher than the mean level in samples from the nonrural area (5 pg/g fat; 1–16 pg/g fat; p<0.0001) and is 10-fold higher than the mean TCDD level for 33 countries (3.4 pg/g fat) (15)
- TCDD was the major contributor to the I-TEQ in the rural (75%) and nonrural (50%) regions
- The correlation patterns of PCDDs/PCDFs in the rural and nonrural regions support TCDD as the major and distinctive PCDD/PCDF contaminant of the rural region
The mean PCDD/PCDF I-TEQ level in breast milk samples from the rural area (46 pg/g fat; range 12–133 pg/g) is higher than the mean level in samples from the nonrural areas (11 pg/g fat; range 4–23; p<0.0001) and higher than the mean level for 33 countries (20 pg/g fat) (/5).

- The consumption of cottonseed oil and kefir positively correlated country-wide with TCDD levels in breast milk; however, in the rural region, only kefir consumption correlated with TCDD levels.
- The PentaCDD/TCDD ratio was 0.5, about four- to sixfold lower than ratios (2–3) typical for industrialized European nations (/5).
- The overall PCB-TEQ (22 pg/g fat) for Kazakhstan was similar to European countries (20–30 pg/g fat), with significantly higher levels in Atyrau (46 pg/g fat).
- Among the six primiparae with TCDD levels >30 pg/g, five had male infants. Selection bias may be operating in the donor population; donors with male infants may preferentially volunteer to participate.
- Studies are under way to determine the prevalence, source(s), and effects of high TCDD exposures in southern Kazakhstan.

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