RISK FACTORS FOR HIGH TCDD LEVELS IN WOMEN FROM COTTON-GROWING STATE-FARMS IN SOUTHERN KAZAKHSTAN

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Introduction

In 1994, the first comprehensive investigation of persistent organochlorine contaminants in a country of the former Soviet Union 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 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 (*1-2*). A follow-up study in 1997 measured levels of PCDDs/PCDFs in samples from the region's cotton-growing State Farms *(4)*. Risk factors for TCDD exposure were evaluated using results from the 1997 study, and are described here.

Materials and Methods

Study design. Procedures used in the earlier studies (design, exposure assessment questionnaire, informed consent, PCDD/PCDF target analytes, WHO/EURO protocol for breast milk sample collection, and statistical analysis) were followed *(1-2)*. As per the WHO protocol *(5),* breast milk donors were healthy primiparae with healthy infants 2-8 weeks of age. Study participants were recruited from a list prepared by the Maternal and Child Health Clinic (MCHC) of all 500 infants in the region that were 2-8 weeks of age at sampling date. 202 of these infant/mother pairs lived on one of the six cotton-growing State Farms that were selected for study because of high birth and infant mortality rates. All of the 59 first-time mothers were recruited, and 41/44 (93%) of available breast-feeding primiparae were enrolled in the study. Multiparae (23 of 143) with adverse reproductive health outcomes (miscarriages, birth defects, or infant deaths) which might signal high TCDD levels were enrolled to help identify risk factors for exposure. Breast milk samples (100 mL) were collected from 64 donors (41 primiparae) from the six State Farms. Donors gave samples of at least one of the following lipid-rich foods: cow or camel's milk, butter, hard white cheese, lamb fat, or cooking oils (cottonseed, sunflower, and bausak). An Exposure Assessment Questionnaire queried demographic, health, diet, breast-feeding, and residential, occupational, and reproductive histories. Food frequencies were self-reported and subject to recall bias.

Three contiguous State Farms with high mean levels of TCDD in breast milk were designated in Zone A, adjacent to a reservoir receiving agricultural runoff. State Farms located farther (>10 miles) from the reservoir were designated in Zone B. "Urban" refers to major cities in southern Kazakhstan (pop 200,000-1 million), distant from the Zone A geographic region. "Rural" refers to the two county seats of Zones A and B (villages of 20-30K). Zones A and B donors are from the same region as "rural" donors, but have lower socioeconomic status and grow their own food.
Analytical methods. Breast milk and food samples were analyzed for PCDD/PCDFs by laboratories at the Centers for Disease Control (CDC) and the U.S. Food and Drug Administration (USFDA), respectively, using HRGC/HRMS (*6)* and quadrupole ion storage tandem MS *(7)*. Residue levels are expressed as pg/g milk lipid. For all reported data, I-TEQs for PCDDs/PCDFs are based upon the WHO-TEQ system *(8)*.
Statistical analysis. 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). Only measurements above the detection level were used. The t-test was used to compare congener concentrations among primiparae. After eliminating co-linear variables, multivariate techniques were used to evaluate risk factors for TCDD exposures.

Results and Discussion

Major risk factors included residence, diet, and pesticide use. Parity and months-lactation were controlled by study design. Highest TCDD levels (up to 208 pg/g fat) were measured in breast milk samples from State Farms in Zone A, and were significantly higher than levels in Zone B (Zone A: mean 53 pg/g, n=17; Zone B: mean 21 pg/g, n=24; p = 0.0017). Zone A levels were 20-fold higher than "background" levels found elsewhere (mean of 33 countries = 3.4 pg/g) *(9)*.

Most (85%) food samples from State Farms were contaminated, suggesting that TCDD exposures have been chronic, environmental, and long-term. TCDD levels in food and breast milk samples followed the same order: State Farms > rural > urban, with levels in State Farms 10-fold higher than US levels (Table 1). Eating contaminated food is the likely TCDD exposure pathway.

Table 1. TCDD levels in foods

Region	n	% Positive	x U.S. levels
State Farms (1997)	45	84	10
Rural villages (1994,1996)	13	58	5
Urban (1994)	7	14	1

Most donors picked cotton as teen-agers, but years-cotton-picking did not correlate with TCDD levels, and some donors with high TCDD levels did not pick cotton. Other findings link TCDD body burdens and pesticide use. First, TCDD and β-hexachlorocyclohexane (β-HCH) levels in breast milk were correlated, and the correlation strengthened in regions with higher TCDD levels: Zone A State Farm 1 > Zone A > all (Table 2). β-HCH is the most persistent isomer of the HCH insecticide mixture (α,β,γ,δ-isomers) that is used locally on sheep. Mean β-HCH levels in the region are 10-fold higher than levels in Europe (1933 vs 200 ng/g fat) *(10)*. Second, the only donor in the cohort reporting active use of pesticides (she drove a tractor sprayer) had unusually high TCDD levels for a woman of her age and parity (Table 3).

Table 2. Correlation between TCDD and β-HCH

Region	n	Correlation coefficient	p-value
Zone A State Farm 1	6	0.81	0.05
Zone A	19	0.49	0.03
Zones A and B	58	0.29	0.03

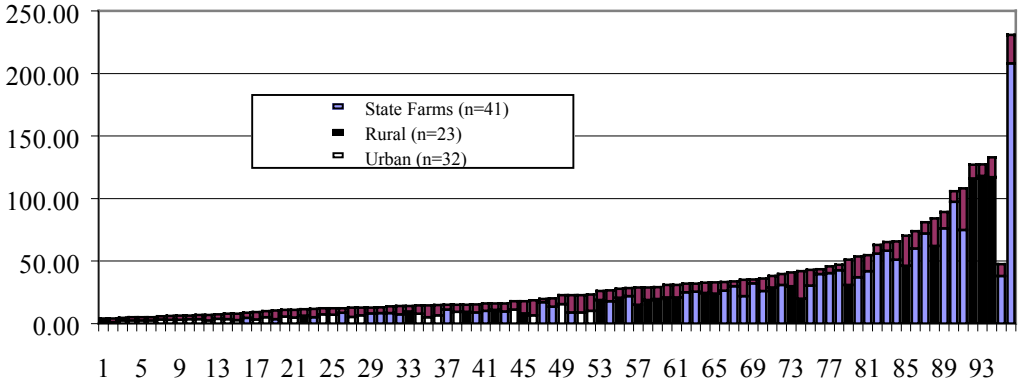
Table 3. TCDD levels in older multiparae

Age	Parity	TCDD (pg/g fat)
36	5	17
37	5	14
37	6	9
40*	6	50

* tractor driver

Finally, the PCDD/PCDF congener pattern is quite unusual: TCDD is the major (70%) contributor to the I-TEQ, compared to 17% of the I-TEQ in 33 other countries (Figure 1) *(9)*. TCDD levels were greater than OCDD levels in Zone A *(4),* whereas they are 1/50th the OCDD levels in other countries *(9)*. The congener pattern resembles that of the TCDD-contaminated herbicides and defoliants, 2,4,5-trichlorophenoxacetic acid (2,4,5-T), and Agent Orange (2,4,5-T/2,4-D, 1:1).

Figure 1. Unusual congener pattern: TCDD vs. non-TCDD congeners in individual primiparae



Cotton was first grown in the region in the early 1950's, with heavy application of pesticides and defoliants by aerial spraying during the period 1965-1985. Defoliants are typically used on cotton in late summer, when temperatures reach 40° C and surface volatilization of pesticides is substantial. Cotton is harvested by teen-agers, and donors describe being sprayed while picking cotton or at home. Health professionals reported over-spray incidents of State Farm communities, followed by outbreaks of headaches and nausea.

TCDD-contaminated stocks of 2,4,5-T and 2,4,5-trichlorophenol were produced in Russia in the 1960's. The only evidence of their use in this region is the distinctive TCDD-dominating congener pattern in breast milk. If TCDD-contaminated pesticides were used on cotton, residents of State Farms have had exposures to TCDD for many years via multiple pathways (e.g. dust inhalation, skin contact with leaves and soil, and ingestion of home grown vegetables, milk, eggs, and meat, and local fish). Future studies will examine TCDD levels in fish, bivalves, and sediment from the adjacent reservoir and irrigation canals in an effort to identify and prevent exposures among State Farm populations to major sources of TCDD contamination.

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