# SEX RATIO OF INFANTS IN A TCDD-CONTAMINATED REGION IN SOUTHERN KAZAKHSTAN

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## Introduction

High levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were measured in breast milk samples collected from first-time mothers ("primiparae") living in a cotton-growing region in southern Kazakhstan (*1-2*). Highest TCDD levels (mean 53 pg/g fat; n=17) were found in State Farms in Zone A, adjacent to a reservoir which receives agricultural runoff from cotton fields *(3)*. TCDD was the dominant congener and the major (70%) contributor to the I-TEQ. This unusual congener pattern resembled that found in persons exposed to the TCDD-contaminated herbicide and defoliant 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). TCDD-contaminated stocks of 2,4,5-T were produced in Russia in the 1960's, and defoliants were applied on cotton in southern Kazakhstan by aerial spraying between 1965-85. Breast milk donors report being sprayed while picking cotton as teen-agers; health workers describe past incidents of aerial over-spraying of cotton-growing State Farm communities, followed by outbreaks of headaches and nausea. The lipid-rich foods (dairy, meat) in these State Farms are widely (85%) contaminated *(3)*. Dietary data, coupled with the history of pesticide use, suggest that exposures to TCDD have been chronic, environmental and long-term. The effect of these presumably sustained TCDD exposures on sex ratio is examined in infants of women living in the TCDD-contaminated region.

## Materials and Methods

***Study design.*** Analytical and questionnaire data were used from the 1997 exposure assessment study of PCDD/PCDFs in breast milk samples from donors living on cotton-growing State Farms in the contaminated region *(3)*. As per the WHO protocol *(4),* most (41/64) breast milk donors were healthy primiparae with healthy infants 2-8 weeks of age. Twenty-three multiparae were also enrolled. 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, born either to primiparae or multiparae. Home births and midwifery do not occur, so all 2- to 8- week-old infants born in the region were listed, along with their mothers' reproductive history (parity, numbers of miscarriages, malformed babies, or infant deaths). 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 contributed breast milk (100 mL) samples. Multiparae (23 of 143) with adverse reproductive health outcomes (miscarriages, birth defects, or infant deaths) which might signal high TCDD levels were enrolled to identify risk factors for exposure.

An Exposure Assessment Questionnaire queried demographic, health, diet, breast-feeding data, as well as residential, occupational, and reproductive histories. 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. Donors from State Farms in Zones A and B are from the same region as "rural" donors from earlier studies (1994, 1996), 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 *(5)* and quadrupole ion storage tandem MS *(6)*. Residue levels are expressed as pg/g milk lipid.

***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.

**Results and Discussion**

Results from earlier studies suggested that higher levels of TCDD were associated with an increased proportion of male offspring. Among primiparous donors whose breast milk was sampled in 1994 or 1996 *(2-3),* women with TCDD body burdens of >30 pg/g fat had mostly (7/8) male offspring (Table 1). The TCDD levels for these donors were 31, 46, 62, 116, 117, and 118 pg/g fat. Among primiparae sampled in 1994, 1996, or 1997, women with TCDD body burdens of >70 pg/g fat had mostly (8/11) male offspring (Table 1). This contrasts with the finding at Seveso, Italy. Here parents with much higher TCDD body burdens (104-2340 pg/g fat) had mainly female offspring. During the eight-year period following the 1976 explosion at Seveso, 9 sets of parents with the highest TCDD levels (archived serum samples since 1976: fathers: 104-2340 pg/g fat; mothers 238-1650 pg/g fat) had 12 female and no male infants *(7)*.

**Table 1. Sex ratio of first-born infants**

| Studies | TCDD (pg/g fat) | M | F | p-value* |
|---|---|---|---|---|
| 1994, 1996 | >30 | 7 | 1 | 0.07 |
| 1994, 1996, 1997 | >70 | 6 | 3 | 0.51 |

*binomial probability test whether the sex ratio is significantly different from 0.514

To more rigorously examine effects of TCDD body burdens on infant sex ratio in the contaminated region in Kazakhstan, we restricted our assessment to the 1997 study population, in which all live births (500) in the region 2-8 weeks before sampling were listed by the MCHC. Mothers were primiparae and multiparae. The MCHC list was comprehensive, and captured all successful births in the region during the 6-week period. The list accurately describes the proportions of male and female infants born during this period. As is seen in Table 2, roughly

equal numbers of male and female infants were born in the overall region (Zone A and Zone B), and slightly more males than females were born in Zone A, the region of higher contamination. In the two major State Farms in Zone A with the most difficult living situations, there were more male than female infants born. None of these alterations were statistically significant. In summary, no significant effects were seen on sex ratios in infants either from the entire contaminated region or in Zone A, the region of higher contamination, nor was any significant effect seen among infants from the 3 major State Farms in Zone A, or from State Farm 1, which had the highest TCDD body burden (Table 2).

**Table 2. Sex ratio of infants 2-8 weeks of age from cotton-growing region (1997)**

| Region | M | F |
|---|---|---|
| Zones A and B | 252 | 248 |
| Zone A | 140 | 120 |
| Zone A 3 major State Farms | 55 | 46 |
| State Farm 1 | 19 | 15 |
| State Farm 2 | 29 | 21 |
| State Farm 3 | 7 | 10 |

To examine the role of the mother's TCDD body burden on infant sex ratio, we used first-born infants from the 1997 study, where primiparae were systematically recruited and participation rates were high. We believe that selection bias for infant gender was minimal, and that the gender profile of first-born infants in the study population accurately reflects the sex ratios of first-born infants from the various sampling sites. In the 1994 and 1996 studies, primiparae were not as systematically recruited, and cultural biases (male children are favored) may have elevated the proportion of male infants in the study over that found in the population sampled. Multiparae were likewise not systematically recruited, and were not used to examine the relationship between TCDD body burdens in women and the proportion of males in their offspring.

Sex ratios were examined for first-born infants in the 1997study population by place of residence and by TCDD body burden of the mother (Table 3). With regard to residence, in the region with higher TCDD levels (Zone A), more male than female infants were born to primiparae during the designated 2-8 weeks. As with the 1994 and 1996 studies, the results were not statistically significant (Table 3). Sex ratios were similar among infants born to primiparae with higher ($\geq$ 50 or $\geq$ 30 pg/g ) or lower ($<$ 30 pg/g) TCDD body burdens (Table 3). With regard to TCDD body burdens, no significant association was seen between TCDD levels and infant gender ($\chi^2$ p=0.83).

**Table 3. Sex ratio of first-born infants (1997)**

| | M | F | p-value | Mean TCDD |
|---|---|---|---|---|
| A. Residence | | | | |
| Zone A (n=17) | 12 | 5 | 0.15 | 53 |
| Zone B (n=24) | 11 | 13 | 0.68 | 21 |
| B. TCDD level (pg/g fat) | | | | |
| ≥ 30 (n=19) | 11 | 8 | 0.65 | |
| < 30 (n=22) | 12 | 10 | 0.83 | |

We believe this population has had a long-term (20-30 year) exposure to TCDD at relatively stable levels, in contrast to the exposure scenario at Seveso. We continue to examine the relationship between TCDD and infant gender in the study populations. Future studies will examine sex ratios of infants retrospectively, using medical records of the local MCHCs, during periods when TCDD contamination was believed to be the highest.

**Acknowledgements**
We express our appreciation to the women of Kazakhstan who volunteered for this study. We also thank G Lucier, D Patterson and W Turner for technical assistance. This study was funded in part by the Trout Farm Ltd. and the National Institute of Environmental Health Sciences.

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