REPRODUCTIVE OUTCOMES STUDY IN WORKERS WITH PAST EXPOSURE TO DIOXINS AT UFA KHIMPROM PLANT

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Introduction

The polychlorinated dibenzo-p-dioxins (dioxins) and polychlorinated dibenzofurans (furans) are ubiquitous environmental pollutants which have significant toxic, immunotoxic, endocrine disruptive, developmental, and carcinogenic effects in laboratory animals. In 1949, the first descriptions of human exposure to 2,3,7,8-tetrachlorodibenzo- p-dioxin (2,3,7,8-TCDD)- contaminated chemicals were reported after a trichlorophenol reactor explosion in Nitro, West Virginia, USA.[1] Reported non-cancer health effects included a range of conditions affecting most systems. The majority of effects have been reported among highly exposed groups including occupational populations, such as chemical production workers, pesticide applicators , and individuals who handled or were exposed to materials treated with 2,3,7,8-TCDD- contaminated pesticides, and among residents of communities contaminated with tainted waste oil (Missouri, USA) and industrial accident (Seveso, Italy). Reproductive health effects of these compounds have been studied in experimental animals and in occupationally and environmentally exposed human populations. While experimental studies have demonstrated a variety of adverse reproductive outcomes, human data are most intriguing for observations on lowering of the male: female sex ratio at birth. Lowered sex ratios at birth have been observed among cohorts of workers exposed to dioxins and among environmentally exposed cohorts.[2,3]

The state agrochemical plant called Khimprom in Ufa, Bashkortostan, in the Russian Federation, was operated since the early 1940s. From 1961 to 1988, over 600 workers in this chemical factory produced 2,4,5-trichlorophenol (TrCP) that was used as a wood preservative and as an intermediate in the further production of both the germicide hexachlorophene and the phenoxy herbicide 2,4,5-trichlorophenoxy acetic acid (2,4,5-T). More than 250 workers produced 2,4,5-T itself for about two years, between late 1964 and the end of 1967. The cohort of workers who were engaged in the production of trichlorophenol and trichlorophenoxyacetic acid at the Khimprom have some of the highest occupational exposures to dioxin reported to date. The Khimprom cohort is also unique in its high percentage of female workers (up to 40%). With funding from a NIOSH and Fogarty International Center grants we have established partnerships with Ufa occupational health researchers and have recruited 560 former Khimprom plant workers for participation in our study of reproductive health in these workers.

The primary hypothesis of the study is that the sex ratio of offspring of Khimprom workers will be significantly lower (i.e. higher percentage of female births) than that of general population controls. Secondary hypotheses in this study is that these occupational exposures adversely affected fertility in men and women, and reproductive outcomes, such as preterm birth, stillbirth, neonatal death, low birth weight and in utero growth retardation.

The specific aims of this study were to: identify, track, and recruit a cohort of workers with occupational exposure to chlorinated phenols, chlorphenoxy herbicides, and their CDD and CDF contaminants at the Khimprom plant from 1961 to 1989; Assess the degree of occupational exposure to these compounds by reviewing length and type of exposure for individuals in the cohort, plant industrial hygiene records, chloracne registry data, and (for a subset), blood levels of CDDs and CDFs; Identify and recruit a comparison group; administer a reproductive outcomes questionnaire to male and female members of the exposed cohort and comparison group and to the spouses of males

in both groups; review vital statistics records (birth and death records) and birth medical records of offspring of both groups; analyze the data to investigate the primary and secondary hypotheses

In this paper we would like to report the data on the sex ratio of offspring fathered by exposed Khimprom plant chemical workers. These data includes only live birth pregnancies and excludes twin pregnancies and pregnancies fathered by men who had multiple marriages and partners.

Materials and Methods

The design of this study is a historical cohort reproductive outcomes study using a comparison population of working controls. The exposed cohort was identified based on the following criteria: any worker who was previously employed for one or more days at the Khimprom plant from the period of January 1960 trough 1987 and who met one or more of the following criteria: worked in Department #5 (Building 50) during the production of TCP and TCP-Cu at any time between 1960 and 1974;worked in Department #50 (Buildings 83 and 83-a) during the production of TCP and TCP-Cu at any time between 1975 and 1987; worked in Departments #19 during the production of the butyl ester of 2,4,5-T at any time between November 1964 and 1968; Worked in the Departments #2, 3 and 11 during the production of 2,4 D at any time between 1960 and 1987; worked in the chemical laboratories during the experimental production of 2,3,7,8 TCDD at any time between 1982and 1984; appeared on the Chloracne Registry**.** Based on these selection criteria we identified, tracked and recruited a cohort of 560 Khimprom plant workers with occupational exposure to chlorinated phenols, chlorphenoxy herbicides, and their CDD and CDF contaminants at the Khimprom plant who worked at the Khimprom plant from 1961 to 1987 (the main group).

Based on data from the Environmental Research and Protection Center of the Republic of Bashkortostan the city of Ufa could be divided into contaminated and uncontaminated territories based on TCDD soil concentration and blood TCDD levels in residents. [4] Based on soil contamination levels, the city of Ufa could be divided into the following 4 zones: **Zone F**: Khimprom plant-1.4 km^2 soil 2,3,7,8 TCDD - 10-200 ppb and blood TCDD levels in workers: mean 267 ppt (1992), 125 ppt (1997); **Zone G**: Industrial zone around the Khimprom plant- 5 km^2 soil - 10-200 ppt and blood dioxin level in residents: 27 ppt (2004); **Zone H**: district of Ufa "increased pollution zone", >300,000 residents - 134 km^2 soil - 3-9 ppt and blood dioxin level in residents: 16 ppt (1998-2004); and **Zone K**: background Ufa, 800,000 residents, soil - 1.7 ppt and blood dioxin level in residents: 12 ppt (1998-2005); TEQ PCDD/Fs: 27 ppt. We selected **our referent population from Zone K**, and identified several enterprises from this zone. Workers from these enterprises agreed to participate in our study. The referents were eligible for selection and recruitment if they have worked in these plants, never worked at Khimprom, a household member never worked at Khimprom, and not resided or worked in contaminated districts of Ufa. The referents were matched to the exposed on age (mean age of current participants is 64 years) gender, and employment status.

Exposure data

Data on exposure and work history for exposed group was available from several sources: 1) the Exposure database of the Environmental Research and Protection Center of the Republic of Bashkortostan; 2) the Health database of the Ufa Scientific Research Institute of Occupational Medicine and Human Ecology on chloracne patients; 3) data from the plant on work histories of employees. We supplemented the above sources of exposure data by administering an Occupational and Environmental History Questionnaire in a face-to- face interview by a trained interviewers who were blinded to the participant's health status. Each participant was asked to respond to an Occupational and Environmental History Questionnaire, developed by the PI (DI) on the basis of the University of Illinois Survey Research Laboratory Questionnaire, and questionnaire developed by Dr. Kruglov and Amirova from the Environmental Research and Protection Center of the Republic of Bashkortostan with the support provided through our Fogarty ITREOH Grant to the UIC Great Lakes Centers. The purpose of this questionnaire was to obtain information on work history independent of the other sources, and to estimate error rates of initial data .

Collection of reproductive health status data
Worker and referent reproductive health status was assessed through an interviewer-administered Reproductive History Questionnaire, and a review of ob/gynecological and vital records. A lifetime reproductive history was obtained from each participant by interviewers blinded to the exposure status of the respondents. The questionnaire covered demographic and reproduction related questions. The demographic portion of the questionnaire covered the date and place of birth, nationality, years of formal education completed. Participants were asked to provide information on their outpatient and obstetrics and gynecological clinics (female participants and male participants' spouses), provide basic data on pediatric polyclinics of their children (including number and location), and to sign a release of medical information from providing study investigators access to all medical records.

Data keying, coding, editing and entry
Data keying, coding, editing and entry were performed at the UIC Data Management Center in Kiev, Ukraine. The quality control of data collected was performed by PI (DI) in Ufa and by the Data Management Center in Kiev

Data Analysis
The presented data on pregnancies fathered by workers fell into two categories: 1) pregnancies fathered by exposed Khimprom plant chemical workers whose spouses never worked at the Khimprom plant; 2) pregnancies fathered by exposed Khimprom plant chemical workers whose spouses worked at the Khimprom plant Sex ratio analysis was limited to live births and did not include pregnancies with twin pairs. The data presented in this article also excludes data from fathers who had multiple marriages and partners. At present we are processing data on our comparison group, thus in this article we compared the observed sex ratios of worker offspring to referent offspring data presented in NIOSH study.[00] Differences between the proportions of boys and girls were calculated with the *z*-test for ratios and X^2 analysis of contingency. The *z*-test was performed with both two-tailed and one-tailed tests with the null hypothesis being differences in the ratio of boys between the exposed population and the referent population.

Results and Discussion
The reproductive health questionnaire was developed and administered to the main and comparison groups. By December 1, 2005 we interviewed 492 subjects from the main group, among them 275 men and 217 women, and their spouses. Among pregnancies fathered by the workers who were married only once and did not have other partners there were 261 eligible live births. One hundred ninety one (199) live births were registered among couples where only men worked, and 62 live births were registered among those couples where both parents worked at the Khimprom plant. The sex ratio of the 261 children fathered by the male workers is presented in Figure 1. We compared the sex ratio of the offspring from the main group to the data on sex ratio of the reference group from the US NIOSH study[5]

Table 1. Sex ratio of offspring of exposed workers

Categories	No. of Children			Sex Ratio (95% CI)	OR (95% CI)
	Males	Females	Total		
Reference category (NIOSH study)[5]	352	295	647	0.54 (0.51 - 0.58)	1
Total Worker Pregnancies	118	143	261	0.45 (0.39 – 0.51)	0.69 (0.52 - 0.92)
Male only worked	95	104	199	0.48 (0.41 – 0.55)	0.77 (0.56 - 1.05)
Both worked	23	39	62	0.37 (0.25 – 0.49)	0.49 (0.29 - 0.85)

The data presented in the current paper shows that in case of pregnancies fathered by exposed Khimprom plant chemical workers whose spouses never worked at the Khimprom plant, the sex ratio of offspring was 0.48, 95% CI, 0.39-0.51. In case of pregnancies fathered by exposed Khimprom plant chemical workers whose spouses worked at the Khimprom plant, the sex ratio was significantly lower than that of the reference group (0.37, 95% CI, 0.25-0.49 and 0, 54, 95% CI, 0.51-0.58, respectively). Worldwide the ratio of live-born male to total births in the general human population is remarkably constant, fluctuating around 0.51 (104–106 boys/100 girls) even when changes are noted with large sample sizes or over long periods of time. The earlier presented data on sex ratio of offspring of men occupationally exposed to TCDD in a pesticide manufacturing plant in Ufa with the median level of TCDD of 240 ng/kg, showed that the ratio of boys to girls at birth was 0.40; this effect was seen only in relation to parental exposures.[2] Studies of the exposed population in Seveso, Italy, also indicate that an increase in TCDD exposure during pregnancy was associated with a substantially lowered male:female ratio (0.38) at birth in the offspring.[3] However study performed by the National Institute for Occupational Safety and Health which compared living workers employed in the production of sodium trichlorophenol (NaTCP), and 2,4,5-trichlorophenoxyacetic ester (2,4,5-T ester) with an unexposed comparison group did not find an association between paternal serum TCDD level and sex ratio of offspring in the population.[5] The chemical exposure of the Russian workers to dioxins was higher than in both the Seveso incident and the U.S. workers. However, all three cohorts have substantial elevated exposure to TCDD and related compounds. The exposure to TCDD is the most likely cause of the altered sex ratio in Khimprom plant workers. Studies suggest that 2,3,7,8-TCDD modulates the concentrations of numerous reproductive and thyroid hormones and/or their receptors.[6] The plausible mechanism responsible for dioxin alteration of level of reproductive hormones is the ability of dioxins to influence the Ah receptor, to which 2,3,7,8 – TCDD binds. Further analysis of our reproductive outcomes data could provide additional information on reproductive outcomes in workers with substantial exposure to TCDD.

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