

# Sex Ratios of Children of Russian Pesticide Producers Exposed to Dioxin

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We investigated the sex ratio of children of pesticide workers who produced the biocide trichlorophenol and the herbicide 2,4,5-trichlorophenoxy acetic acid from 1961 to 1988 in the city of Ufa, Bashkortostan, Russia. We measured exposure of the two related cohorts to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and other dioxins by analyzing 84 blood samples, which produced median TCDD toxic equivalents blood lipid values of 240 ng/kg, which are more than 30 times higher than background or normal exposure from the region. The sex ratio (fraction male) of the combined cohort of 227 children from 150 male and 48 female workers was 0.40, significantly lower (*z*-test for proportions = 3.21; *p* < 0.001) than those for the city of Ufa (0.512) and elsewhere worldwide. When we analyzed the sex ratio of the children according to maternal or paternal exposure, we observed a decrease in the number of boys (ratio 0.38) for fathers and a normal number (ratio 0.51) for the mothers. Human exposure of these pesticide producers to high levels of dioxins is associated with the birth of more girls, but only for paternal exposures. **Key words:** dioxin exposure, Russian chemical producers, sex ratio. *Environ Health Perspect* 110:A699–A701 (2002). [Online 10 October 2002]

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Workers who produce chlorine-containing chemicals have always been at risk from exposure to and health effects of the persistent and bioaccumulative microcontaminants chlorinated dibenzo-*p*-dioxins and furans. Historically, the effects of dioxin-like compounds focused on their acute toxicity and carcinogenicity, but lately the more subtle and sensitive effects of altered development and reproduction have been recognized (Schecter 1994; U.S. EPA 2000). This reasoning has been fortified by the report (Mocarelli et al. 1996) of a skewed offspring ratio (65% girls) from individuals highly exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) as a result of an accidental exposure at Seveso in northern Italy in July 1976. Further study of the Seveso cohort revealed that the effect was associated with paternal, not maternal, TCDD exposure (Mocarelli et al. 2000). However, this biased sex ratio has not been consistent among exposed cohorts. Moshhammer and Neuberger (2000) reported a small difference of 0.46 in an Austrian worker cohort; Rogan et al. (1999) found a slightly higher value of 0.49 in the Yu-cheng rice oil victims; and Michalek et al. (1998) found no effect for this outcome in the lowly exposed Ranch Hand cohort by American Vietnam veterans who sprayed Agent Orange. In a conflicting study, no effect on the sex ratio was detected in a recent publication (Schnorr et al. 2001) from producers of pesticides in two U.S. cohorts that had high TCDD exposure.

The city of Ufa is situated at the eastern edge of Europe in the Bashkortostan Republic of Russia, just west of the Ural Mountains. It was the site of one of the state agrochemical

plants called Khimprom, which has 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. Most of the workers were young men in their early twenties and recently graduated from technical school, but about 15% of the 2,4,5-T and 30% of the TrCP work force were young women.

In September 1992, about 25 years after the 2,4,5-T herbicide production had ceased, blood sampling and analysis of about 60 men, women, and children from this factory and the city of Ufa took place. For the 2,4,5-T producers, the results showed high exposure (Ryan and Schecter 2000) to both TCDD and significant amounts of the equally toxic 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PnCDD). The TCDD blood concentrations were more than 30 times greater than contemporary blood values from the Bashkortostan region. Blood dioxin levels for the TrCP workers were originally obtained from a small sampling in 1992 which indicated a higher overall toxic equivalent (TEQ) exposure along with the presence of the toxic PnCDD congener. We now describe further blood measurements of these two cohorts which have been made in the intervening years by the Bashkortostan Republic Environmental Centre (BREC). Using these additional blood data and in light of the positive association of high dioxin values on a skewed sex ratio for

the Seveso cohort in Italy and a lack of such finding in other dioxin cohorts, we report also on the sex ratio for the highly exposed Ufa Russian chemical workers.

## Materials and Methods

**Blood lipid exposure.** In addition to the more than 60 samples obtained in 1992 whose dioxin values were reported previously (Ryan and Schecter 2000), further whole blood samples were obtained with consent from the cohort workers. These included 20 samples from the 2,4,5-T group taken in 1997–2000 and 23 samples collected in 1997–2001 from the more numerous and longer existing TrCP cohort. These blood samples were analyzed by BREC using sample enrichment and isotope dilution high resolution mass spectrometry techniques similar to those used by Ryan and Schecter (2000). Both laboratories have participated in World Health Organization-sponsored interlaboratory studies to check the reliability and consistency of human PCDD/polychlorinated dibenzofurans (PCDF) determinations. In addition, both laboratories have successfully exchanged laboratory biota samples and compared results as part of normal quality control laboratory procedures.

**Workers and their children.** Company archival records of the individual workers were consulted for age, sex, time of working in the designated shop, and number, sex, and date of birth of children. Verification of company information and additional data on date of birth and sex of all live-born children were obtained by interview either by personal contact or by telephone with each worker or a close relative. Through company information and public meetings, workers from whom blood was not obtained or requested were aware of the health outcome studies. The number and sex of live children born any time after 9 months for which the worker was first employed in either of the two factory shops were used for calculation. Any children born before the parent's employment in either shop were excluded from analysis. Also included were data on any offspring who were born live but were deceased in the interim. Most of the children were born between 1965

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and 1972, with smaller numbers born through 1991. The sex ratio or fraction male is the number of males (M) divided by the total number of male and female (F) children—i.e.,  $M/(M + F)$ . Comprehensive data on the number and sex of live births in the years 1959–1996 from the city of Ufa and the Republic of Bashkortostan were available from the State Regional Statistical Department of the Republic. Birth numbers from five individual years approximately spaced between 1959–1996 were averaged for the city of Ufa (34,132 males; 32,563 females; proportion males, 0.512; 105 M/100 F) and used as the basis for comparison to those obtained from the workers. The Republic of Bashkortostan, much larger in population, showed virtually identical sex ratios for the same time period.

**Statistical treatment.** Descriptive statistics (mean, median, variance etc) were conducted on the blood data normalized as to lipid content by standard methods using SigmaStat (SPSS Science, Chicago, IL). Differences between the proportions of boys and girls were performed with the  $z$ -test for ratios and  $\chi^2$  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 control population from the city of Ufa.

## Results

Table 1 gives the number of workers in both cohorts, their blood lipid exposure expressed in TCDD TEQs, and the number and sex of their children. The sex ratio is compiled for both cohorts separately and combined, and is further separated between mother and father worker. The new blood data by BREC confirmed the even higher TEQ exposure for the TrCP cohort with the appreciable presence of PnCDD. For both cohorts, more than 70% of the worker blood TEQ originates from TCDD, with significant contribution from the equally toxic PnCDD. The TEQ values from blood samples collected many years after initial exposure are one to two orders of magnitude higher than background or normal concentrations from this region or elsewhere worldwide, attesting to the overall elevated exposure to dioxins. The blood lipid TEQ values for the TrCP cohort are more than three times higher than the 2,4,5-T group. However, some of this exposure difference is due to *a*) the date of blood sampling, which for the TrCP cohort was closer in time to the actual date of chemical production, and *b*) to the longer time individuals spent in the TrCP shop that manufactured this chemical.

When the  $z$ -test is used for proportions, the ratio of male children for both cohorts (0.40) is statistically different ( $z = 3.21$ ;  $p < 0.001$ ) from normal live births in this region

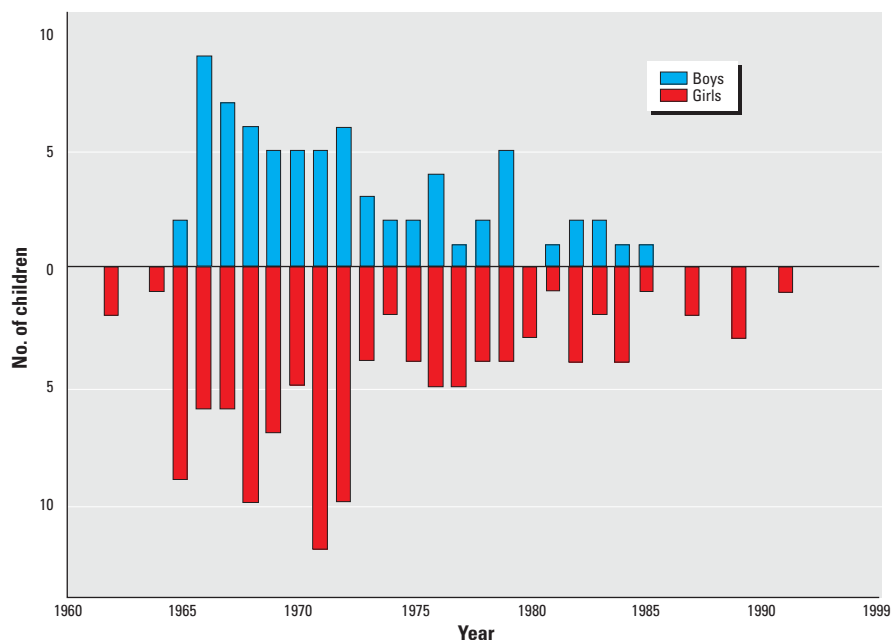
and worldwide (about 105 males for 100 females). Differentiating the children according to either their exposed mother ( $n = 48$ ) or father ( $n = 150$ ) shows a further decrease in the number of boys for the fathers (ratio 0.38) and a normal number (ratio 0.51) for the mothers. The ratio of boys for the fathers only is significantly different ( $z = 3.60$ ;  $p < 0.001$ ) from those of the control populations. The degree of exposure as indicated by the blood lipid values was virtually the same for

the mothers and fathers in both groups. The sex ratio is as low as 0.23 (7 M/23 F) for those fathers from the TrCP cohort with measured blood values (median of 715 ppt TEQ blood lipid). The sex of the 188 children fathered by the 150 male workers is presented in Figure 1. This histogram shows visually the preponderance of girls over boys. Both of these results—the altered sex ratio and the association with the father and not the mother—are identical to what was found

**Table 1.** Numbers, TEQ dioxin exposure, and sex of offspring of Russian chemical producers.

Cohort	2,4,5-T	TrCP	Combined
<b>Workers</b>			
No. for whom birth data available/total	88/> 250	110/> 600	198/> 850
Sex (M/F) of workers with birth data	(74/14)	(76/34)	(150/48)
<b>Worker exposure</b>			
No. blood samples analyzed	55	29	84
Sex (M/F) of donors	(43/12)	(22/7)	(67/19)
Years since exposure (median)	23	12	—
Years worked in shop (median)	(5.5)	(13)	—
TEQ <sup>a</sup> (median)	177	672	243
(Range)	(17–1,930)	(87–8,520)	(17–8,520)
TCDD/PnCDD values	124/35	40/168	—
TEQ (TCDD mean values)			
Bashkortostan residents		27 (4.8)	
Ufa residents		47 (17)	
<b>Children of workers</b>			
Born during and after worker exposure	126	101	227
No. children by sex (M/F)	52/74	39/62	91/136
Sex ratio (M/M + F) with both parents	0.41	0.39	0.40*
Fathers only ( <i>n</i> )	74	76	150
No. children by sex (M/F)	44/66	27/51	71/117
Sex ratio using fathers	0.40	0.35	0.38**
Mothers only ( <i>n</i> )	14	34	48
No. children by sex (M/F)	8/8	12/11	20/19
Sex ratio using mothers	0.50	0.52	0.51
Sex ratio Ufa city		0.512	
1959–1996; <i>n</i> = 66,700 exposed children		(105 M/100 F)	

<sup>a</sup>Values in nanograms TEQ per kilogram blood lipid (ppt). \* $z$ -test for proportions = 3.21 ( $p < 0.001$ ); \*\* $z = 3.60$  ( $p < 0.001$ ).



**Figure 1.** Histogram of sex of 188 children of 150 exposed male workers over time.

in the Seveso incident (Mocarelli et al. 1996, 2000) and differ from what has been reported for the U.S. chlorophenol cohort (Schnorr et al. 2001).

## Discussion

These results, comprising relatively large numbers of blood exposure values and sizeable numbers of offspring, show that the Ufa workers produced an excess of female children associated with their fathers' exposure to dioxins. Worldwide the ratio of live-born male to total births in the general human population is remarkably constant (James 1996), fluctuating around 0.51 (104–106 boys/100 girls) even when changes are noted with large sample sizes or over long periods of time (Allan et al. 1997). Both the city of Ufa and the surrounding Republic of Bashkortostan have shown this sex ratio in the years 1959–1996. We attribute the exposure to TCDD as the most likely cause of the altered sex ratio. However, in most studies of the association between environmental chemicals and altered sex ratio, humans are exposed to many chemicals. In the Ufa cohort as well as those of Seveso, Italy, and the United States (Schnorr et al. 2001), significant exposure to chlorinated phenols and phenoxy pesticides occurred. These compounds have short half-lives in humans, so a retrospective study of exposure is not possible. However, these less persistent compounds are still candidates as endocrine-disrupting chemicals in these episodes.

In this article we present measured blood data on 84 individuals many years after their exposure in chemical manufacturing. We have not attempted to back-calculate to estimate the blood dioxin content at the time of the workers' employment, when their blood levels would have been considerably higher. These estimates usually have a high degree of

uncertainty because mostly single blood measurements are available and the rate of elimination of dioxins in humans is nonlinear (Carrier et al. 1995) with short half-lives at higher exposure. In addition, the dates of beginning and ending employment vary among individuals, as does possible and unknown dioxin exposure subsequent to employment.

Nevertheless, the effect we show here indicates that dioxins act as endocrine disruptors and cause some type of hormonal imbalance. A plausible biologic explanation for the change in sex ratio is not obvious. Mocarelli et al. 2000 have suggested that early age of exposure of the parent may be a factor. We are unable to test this hypothesis in our study because the mean age of the parents at birth of their children was about 29 (range 20–43) years. Other hypotheses that have been suggested to explain the low sex ratio are an antiandrogenic effect of dioxin that alters sperm transit time, with resultant delay of fertilization of the oocyte (Jongbloet et al. 2002). At present, data to support or refute this explanation are limited. Nor can we offer a causal explanation for the consistency of our results with those of Seveso and for their difference with the U.S. cohort. The chemical exposure of the Russian workers to dioxins was probably somewhat higher than in both the Seveso incident and the U.S. workers, when one considers the time of blood sampling and, for the latter cohort, the number of years working. However, all three cohorts have substantial elevated exposure to TCDD and related compounds. A more plausible explanation for the low sex effect in the Yu-cheng poisoning is available. In that episode, exposure was to heat-degraded PCBs containing elevated levels of PCDFs; but the cohort studied was comprised mostly of women, and indications are that maternal exposure is not critical in sex alteration of these environmental chemicals.

The different results among all the described cohorts underline the uncertainty of epidemiology in the study of adverse effects that may be subject to undetected effect modification. Further study of these groups and of related chemicals is necessary for understanding the effect of chemical exposure on human reproduction.

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