

# Chapaevsk, Russia: 40 years of dioxins exposure on the human health and 10 years of Russian –USA epidemiological studies

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## Introduction.

The town of Chapaevsk /population 80 thous/ is located in Middle Volga region. During 1967-1987 a chemical plant there produced hexachlorocyclohexan (lindan) and its derivatives. Later it produced crop protection chemicals (liquid chlorine, acids, methyl chloroform, vinyl chloride, and some other chemicals). Previously it was considered that hexachlorane production was responsible for dioxin contamination in the city's environment. Tests seemed to confirm it. But after the production was stopped in 1987, a continued output of dioxin was still observed. At present the plant stands practically idle; the main contamination source is represented by the vicious technological equipment, the plant's territory and industrial wastes.

In 1994 an average concentration of dioxins in the air was 0.116 pg/m<sup>3</sup>. The calculations were made when the plant worked at 20% capacity, so one can extrapolate that dioxin air emissions had been higher previously. Moving away from the plant one can see the decrease in dioxin levels down to 36.8 ng/kg in downtown (2.7 km from the plant); down to 3.9 ng/kg in the southern part of the city; down to 0.9 ng/kg at 10 – 15 km from the plant. Private houses owners (18,000 in Chapaevsk) grow essentially all their vegetables and fruits for their own use, thus receiving an additional dioxin load. The results received in Chapaevsk boys study show a high proportion of the boys consumed locally grown or raised foods during their lifetime: over 70% consumed locally produced dairy products, over 50% consumed locally raised chickens or eggs, and over 80% consumed locally caught fish during their lifetime [Sergeyev O. et al., 2002].

Dioxins were found in all samples of cow milk: 2,3,7,8-TCDD was 17.32 pg/TEQ/g fat; 1,2,3,7,8-PeCDD was 61 pg/TEQ/g fat. In most samples there were 1,2,3,4,7,8-, 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HxCDD and furans 1,2,3,4,6,7,8-, 1,2,3,6,7,8,- and 1,2,3,7,8,9 –HxCDF detected. The rest of the congeners were on the edge of the detection limit. Dioxin and furan content in the cow's milk is significantly higher than the accepted regulatory standard in Russia (5.2 pg TEQ/g fat). Unfortunately, we do not have data about dioxins content in animals meat /pigs, cows/ being kept in the town, in chickens, in eggs and fish from local rivers and ponds. (Environmental and public health in Chapaevsk city, Moscow, 2000).

## Materials and Methods

In 1994 began studies of dioxins impact on human health with the following aims:

- 1) to estimate dioxins levels in blood and milk ;
- 2) to estimate incidence and mortality rates, and specifically describe reproductive health in the population according to official statistical data;
- 3) to estimate dioxin exposure as risk factor of breast cancer-/case-control study/. MacArthur Foundation Grant
- 4) to estimate dioxin impact on sexual and physical development of boys

Since 1998 a number of joint Russian-American project on Chapaevsk was conducted. The first project was realized in 1998 under the direction of Prof. Paolo Toniolo; it included dioxins study in blood of 24 town residents [Akhmedkhanov A. et al., 2002]. The next pilot project joint with HSPH was conducted in 1999-2000 and it included evaluation of male sexual maturation and abnormalities in the male reproductive system [PI – Russ Hauser (HSPH); Sergeev O. et al., 2000; Lee M. et al., 2002.]. The results of this pilot study led to the development of a proposal for a full-scale study “Dioxins, male pubertal development and testis function.” This proposed project was funded by the EPA for four years beginning 02/2002. The project integrates the efforts of scholars from Harvard School of Public Health, Duke University Medical center, University of California - Davis, Center for Demography and Human Ecology of Institute for Forecasting, Russian Institute of Nutrition, Samara Medical University [PI – Russ Hauser, HSPH].

Besides that, in 2001- 2003 pilot project on study of semen quality joint with R.Hauser (HSPH), J. Overstreet and C.Brazil (University of California, Davis, Center for Health and Environment) and S. Swan (University of Missouri) was conducted; it included an establishment of Andrology laboratory in Chapaevsk for participation in the international multi-center semen studies.

## Results and discussion

**Human milk.** Sampling of human milk was carried out in December of 1997-June of 1998 according to the WHO criteria. Forty samples of human milk were pooled into seven samples. The average age of mothers was 22.0 years. All surveyed mothers classified their diet as mixed. Ninety percent of women eat fish once a week or rarely, eighty percent of women eat meat more than two times a week, and dairy products every day. All respondents never smoked, as they self-reported in the interview. The analysis of dioxins in 7 pooled samples of human milk (40 individual trials) has been carried out. Middle dioxins content in blood was 42,26 pg TEQ<sub>WHO</sub> /g fat.

**Blood.** Blood samples were taken from 14 people. Ninety percent of women lived in Chapaevsk more than 3 years and 75% of them for more than 5 years. Income for one member of a family in these three groups was nearly the same. A detailed inquiry into diet did not find great differences between the groups. A comparison of the dioxin contents in the blood of different groups of the population shows considerably higher levels of dioxin, especially of 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD in female workers' blood. There are distinct differences in the levels of dioxin in women living in two different regions of the town. In four female workers' blood samples - 412,4 pg TEQ/g fat, in six residents' blood samples (those who lived 1-3 km from the chemical plant) - 75,2 pg TEQ/g fat, in four residents' blood samples (5-8 km from the plant) - 24,5 pg TEQ/g fat [Revich et al, 2001].

Other studies on dioxin content in blood made in ?DC had shown rather analogous results. During the summer of 1998, 24 volunteers were recruited to donate blood and to complete a self-administered questionnaire about their residence, employment, demographics, medical history, and dietary habits. Selected polychlorinated dibenzodioxins, dibenzofurans and coplanar biphenyls were measured in blood serum samples. The mean concentration of total dioxin -levels was 61.2 TEQ<sub>WHO</sub> (range 16.4-168.1) parts-per-trillion (ppt). Subjects living in close proximity to the plant (less than 5 km) had significantly higher dioxin levels (mean -TEQ<sub>WHO</sub> 75.7 ppt), as compared to subjects living more than 5 km from the plant (mean WHO-TEQ 44.1 ppt) ( $p = 0.04$ ). Comparison of the study results with available published data revealed that Chapaevsk residents had considerably higher average blood dioxin levels than general populations in other parts of Russia, Europe and North America. The results indicate that residents of Chapaevsk experience substantial non-occupational exposure to dioxins, which could have adverse effects on public health [Akhmedkhanov et al, 2002]. The results of dioxin investigation in blood samples of 30 boys of 14-16 years under the project “Dioxins, male pubertal development and testis function” are presented in the report “Characterization of and predictors of serum dioxin levels among Chapaevsk adolescent boys” submitted to Dioxin2004.

**Breast cancer case-control study.** In 2000 the register of breast cancer patients was developed. All alive women born from 1940 who had breast cancer diagnosis before age of 55 years were included in the study. The response in case group was 97%. A special questionnaire was developed and it included

questions on birthplace, occupation and residence of women and their parents, diet, lifestyle, reproductive function, medical and genetic history. The selection of controls was taken from the general medical insurance database. This group was matched by age only. For the higher reliability of results, the ratio of number cases/controls was 1:2.

Low migration level is well illustrated by the fact that more than a half of respondents were born in Chapaevsk. Mean residence time in the city was the same for 55% cases and controls - near 40 years. As a result of the case-control study conducted with the aim to estimate the risk of breast cancer development for women exposed to dioxins in Chapaevsk City the effect of the following factors was noted:

Type of occupational activities - a significantly higher risk was observed among women mostly engaged in chemical plant, OR =2.1. Mean time of occupation at chemical plant was 20.4 and 12.1 years in cases and controls respectively. It is important that there were no another established professional risk factors in both groups. Not only breast cancer patients themselves were significantly more occupied in chemical production but their parents too comparing with the controls (27.7% and 19.7%). Oral contraception is also significant factor for development of breast cancer OR=2.4. A prior history of benign breast disease occurred 2 times more frequently among cases than among controls: 10.8% and 6.9%, respectively. Fibroadenomatosis and fibroadenoma were noted the most frequently. The presence of at least one full-term pregnancy during lifetime - for childless women the risk of breast cancer development is in 3.8 times higher than for women who have at least one child,  $p= 0.04$ . Presence of breast cancer in genetic relatives increases the subsequent risk of breast cancer development 9-fold,  $p= 0.006$ .

This study demonstrated strong effects of diet habits. Risk increased for women who use more than 50% of pork from farms in the Chapaevsk region (OR=5.7), more than 50% of fish from the nearest lakes or rivers – OR= 2.3. In the case group the usage of pork fat for cooking is significantly higher compared with controls [Revich et al.,2001b]

**Physical and sexual maturation of adolescent boys.** The objectives of this pilot study were: evaluation of physical and sexual maturation and revelation of the prevalence of minor urogenital abnormalities among a sample of adolescent boys (10-16 years old) residing in Chapaevsk [Lee M et al., 2002, Sergeyev O. et al., 2002]. Male children born between 1<sup>st</sup> October, 1982 and 1<sup>st</sup> October 1988 were eligible for examination and were identified through their school. Among 3041 age-eligible boys (age 10-16 years), 2580 (84.9%) were examined in 1999 by a pediatric endocrinologist and an urologist. The questionnaire included questions on diet, lifestyle factors and medical history, such as whether the child was ever diagnosed or treated for undescended testicles. Anthropometric measurements, pubertal maturation and penile and testicles measurement were performed.

Among 2580 children screened for minor urogenital abnormalities and delayed sexual maturation, 45 (1.7%) had cryptorchidism, 18 (0.7%) had hypospadias, 200 (7.8% ) had phimosis or Difficulties with penis head opening, 500 (19.4%) had varicoceles, and, within boys older 14.26 year, 82 (8.7%) had delayed sexual development. Anthropometric data were compared to U.S. reference data for 2000 by computing a z-score for each individual, then calculating the mean z-score within each one-year age interval and an overall z-score. The mean z-scores for height, weight, and BMI as compared to the US data were -0.18, -0.52, and -0.61, respectively, indicating that across all ages, the Russian youths were consistently thinner and slightly shorter than US boys. The greatest height difference was observed at ages 12-14 (z-scores between -0.20 and -0.27). In contrast, the mean weight and BMI (z-scores ranging from -0.27 to -0.69) were consistently lower than the U.S. norms for all age groups.

The mean heights and weights for Chapaevsk boys were also compared to previously published Russian data drawn from a larger geographic area, which included urban youth from the cities of Moscow and St. Petersburg, as well as other urban and rural regions in Russia [Martinchik AN, Baturin AK, 2000]. The Chapaevsk boys were consistently taller but lighter than the general Russian sample. When a weighted regression model was fitted to the mean heights and weights (weighting inversely by the variance), the Chapaevsk boys were on average 1.15 cm taller ( $p=0.01$ ) and 1.28 Kg lighter ( $p=0.06$ )

The first evidence of sexual maturation, stage 2 genitalia development, occurred at a median age of 11.9 years among Chapaevsk boys, which is generally older than that reported elsewhere. For example, the median age of stage 2 genitalia reported recently from East Germany is 10.8 years, and from the United States is 11.5 years according to data reported by Tanner and Davies in 1985 and 10.1 years for U.S. Caucasian boys according to 1988-1994 NHANES III data. The median age of 12.7 years for stage 2 pubic hair growth in Chapaevsk boys is older than that reported recently from East Germany (11.5 years) and from both the 1985 and the 1988-1994 NHANES III US data (12.0 years). Consistent with the delayed onset of puberty, the attainment of full sexual maturity appears to occur at an older age in the Chapaevsk boys compared with other populations. In East Germany, by 16.99 years of age, greater than 75% boys have attained stage 5 maturation for pubic hair or genitalia, whereas in a comparable age group, less than 20% of the Chapaevsk boys have attained stage P5 or G5. The median age for attainment of full sexual maturity in the Chapaevsk boys, defined as stage 5 genitalia and pubic hair, occurred at 15.4 and 15.6 years, respectively. In conjunction with the trend towards a later onset of puberty and attainment of sexual maturity, the lower mean height in the Chapaevsk boys compared to U.S. boys, particularly at 12 to 14 years of age, is suggestive of an apparent delay in timing of the pubertal growth spurt.

An investigation of the potential association of dioxin exposure with the genital and maturational abnormalities observed in these boys is underway. We have collected and archived blood samples on boys (and their mothers) with cryptorchidism, hypospadias, and delayed sexual maturation as well on control boys.

**Semen quality.** Dioxin exposure may influence negatively to semen quality and hormonal status of men. Last years scientific medical literature consists many discussions about lowering of quantity and quality of men sperm in number of European countries (France, Belgium, Scotland) and in USA, in which connection environmental chlororganic pollution is called as one of risk factors. Semen examination of 51 workers from former chlororganic pesticides production showed statistically reliable lowering of spermatozoa number and change of their normal morphological forms [N.Goncharov et al., 1999]. Study of semen quality in young men which had born in Chapaevsk and live there is planned jointly with HSPH, Andrology Coordination Center at the UC Davis and University of Missouri.

Measures on improvement of the city medical service. The epidemiological research results together with other works on estimation of Chapaevsk environmental pollution have made a basis for Chapaevsk inclusion in the list of the most polluted Russian cities (containing only 11 cities) /disaster/ and for realization of the special program on Chapaevsk health service improvement. New medical equipment was installed in the maternity hospital /incubation apparatus, new-born reanimation system, US-apparatus with dopplerography, fetomonitors/. These and other measures on neonatological aid improvement have resulted to the decrease of infant mortality from 32,0 of 1000 new-born in 1996 to 10,6 in 2003. The medical activity was also directed to treatment of boys with pubertal development impairments, revealed during the epidemiological research. Upward of 100 children were operated for phimosis, varicocele, cryptorchism. All 139 boys with delayed sexual development are observed by endocrinologist, andrologist and urologist, and receive necessary treatment. Screening ultrasound investigation of new-born excretory system on congenital pathology is conducted. The cancer-register is formed on the basis of the oncological cabinet. Informative issues on breast cancer' risk factors and prophylaxis are published for Chapaevsk population. Within the framework of the project "Pilot Studies of Semen Quality in Russian Men" funded by CRDF the modern equipment for andrological laboratory was purchased, that allows to receive necessary quantitative readings of examined men' reproductive health state in the limits of the epidemiological studies.

## Acknowledgements.

This epidemiological work in Chapaevsk would be impossible without permanent support of the town government – the former mayor Yurii Lipchenko and the active mayor – Nikolay Malakhov, their deputies, Direction of the town environmental programme, physicians and nurses of Chapaevsk central hospital, maternity hospital and other medical institutions of the town. Special thanks to population of Chapaevsk. We thank our colleagues – Larisa Althul, Paige Williams, Sussan Korrick from Harvard School of Public Health, Paolo Toniolo, NY U Sc.of Medicine, Mary Lee from Duke Univ. NC, James Overstreet and Charlene Brazil from Inst. of Toxicology and Env. Heath US Davis, Shanna Swann, of Missouri, provided an idea of joint Russian-American projects on study of dioxins exposure on Chapaevsk population health and did everything in their power for obtaining financial support from ???, NIH, NIEHS, NCI, Center of Research and Development Foundation (CRDF). MacArthur Foundation made an important contribution to these works, have being given the grant to breast cancer study.

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