Characterization and predictors of serum dioxin levels among adolescent boys in Chapaevsk, Russia

Russ Hauser[1], Paige Williams[2], Larisa Altshul[1], Susan Korrick[3], Lynne Peeples[2], Donald Patterson[4], Wayman E. Turner[4], Mary M. Lee[5], Boris Revich[6], Vladimir Zeilert[7], Oleg Sergeyev[8]

[1]Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA
[2]Department of Biostatistics, Harvard School of Public Health, Boston, MA, USA
[3]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
[4]Center for Disease Control and Prevention, Atlanta, GA, USA
[5]Pediatric Endocrine Division, Duke University Medical Center, Durham, NC, USA
[6]Center for Demography and Human Ecology of Institute for Forecasting, Russian Academy of Sciences, Moscow, Russia
[7]Chapaevsk Central Hospital, Chapaevsk, Russia
[8]Chapaevsk Medical Association, Chapaevsk, Russia

Introduction

Although toxicological studies have demonstrated an association between exposure to polychlorinated dibenzo-*p*-dioxins (PCDDs) and adverse developmental and reproductive health effects, human evidence is limited. In particular, the health consequences of childhood and adolescent exposure to PCDDs have been inadequately investigated. Given the animal data and the evidence that children may be more sensitive to PCDDs than adults, we conducted a pilot study to determine exposure levels among adolescents living in Chapaevsk, Russia, where environmental levels of dioxin are high. Chapaevsk, a town of approximately 80,000 residents, is 43 kilometers southwest of Samara on the Chapaevsk River, a tributary to the Volga. Half of the town area of 187 km^2 is occupied by industrial manufacturing facilities employing almost half of the city's work-force.

One of the largest factories in Chapaevsk is the Khimprom Chemical Plant, which before 1949 produced chemical warfare agents (e.g. lewisite and mustard gas). Since 1967 the plant has produced pesticides, including gamma-hexachlorocyclohexane (lindane), and chlorine-containing products such as liquid chlorine, dicholoropropionic acid, methyl chloroform, vinyl chloride, and pentachlorophenol[1], generating PCDDs and polychlorinated dibenzofurans (PCDFs) as industrial contaminants, which subsequently polluted the local air, soil, water and food supply[1]. Although the plant has reduced manufacture of these chemicals in the past decade, PCDDs and PCDFs are environmentally persistent compounds, thus raising concern about continued human exposure from contaminated air, soil, drinking water, and locally produced vegetables and animals[2]. Furthermore, a large proportion of the population lives close to the Khimprom complex.

Our pilot study in Chapaevsk was designed to determine the feasibility of studying the relationship of exposure to PCDDs and PCDFs with somatic growth, pubertal development, and hypothalamic-pituitary-gonadal function among peri-pubertal Chapaevsk boys. Aims of the pilot study included identifying potential predictors of serum levels of PCDDs and PCDFs among adolescent boys and assessing the relation of these measures with genito-urinary development and adolescent sexual maturation. Due to the high expense involved in measuring serum PCDDs and PCDFs, these analytes and coplanar and mono-ortho PCBs were measured in serum from a small subset (n=30) of boys participating in the pilot. Potential predictors of these 30 boys' serum dioxin levels and the relationship of dioxin levels with sexual development were assessed. Throughout the remainder of this report, the term dioxins will include PCDDs, PCDFs, and co-planar PCBs.

Methods and Materials

Study Population: From a group of 2579 boys aged 10-16 years enrolled in a previous study of male growth and maturation in Chapaevsk[3], 246 boys aged 14.00 to 16.99 years in 1999 were selected for blood sampling and historical questionnaires. Of the 246 boys, 221 had blood samples collected, and 30 of these bloods were sent to the CDC for dioxin measurements. The thirty blood samples were selected to include fifteen boys with cryptorchidism, hypospadias or delayed puberty (cases) and fifteen boys without these genito-urinary anomolies or delayed puberty (controls). Each boy and his mother completed a questionnaire on medical history, diet, and lifestyle. Diet questions included measures of current and lifetime consumption of locally grown foods. The distance each boy lived from the Khimprom factory was assessed by maternal report and by ArcView GIS 3.0 mapping of addresses.

Dioxin, furan and PCB chemical analysis: Serum was analyzed at the Centers for Disease Control and Prevention (CDC, Atlanta, GA)[4]. Target analytes included PCDDs, PCDFs, non-ortho substituted (coplanar) polychlorinated biphenyls (co-PCBs), mono-ortho substituted PCBs and other PCBs (without assigned TEFs).

Statistical Analysis: Wilcoxon ranksum tests were used to compare dioxin and PCB levels between controls and boys with genito-urinary abnormalities or sexual delay. Generalized linear models were used to identify predictors of the log of the sum of dioxin concentrations and log sum of dioxin TEQs. Predictors of dioxin concentrations were first modeled univariately and then adjusted for age. Since the dataset was small, predictors with p-values below 0.15 were considered to have suggestive associations with dioxins. Robust model selection procedures were utilized to identify appropriate multivariate models, including forwards and backwards selection procedures (retaining only those variables with p-values ≤ 0.15) and Mallows C(p) criterion. Separate models were fit considering all covariates and then considering only covariates collected on all 246 pilot study participants (i.e., excluding PCB 118), to allow prediction of dioxin levels in the larger pilot study cohort. Dietary exposures were coded as yes or no for consumption of food from local sources (own garden or livestock, friend's garden or livestock, or grown/obtained in Chapaevsk) within the following categories: (1) fruits and vegetables; (2) chicken; (3) goats, cows, pigs, and other non-chicken meat; (4) milk and dairy products, including eggs; and (5) fish.
In some analyses, categories (2) and (3) were combined to create a "local meat" consumption group. Multivariate models also considered potential confounders such as socioeconomic status

(family income level, maximum parental education level), reproductive history (gravidity, parity, and total duration of breastfeeding for previous children), and duration of residence in Chapaevsk.

Results and Discussion

Of the 30 boys with serum samples analyzed for dioxins, six had hypospadias, nine had cryptorchidism, and two had delayed sexual maturation. The two boys with delayed maturation had cryptorchidism. Fifteen boys did not have either a developmental abnormality or delayed sexual maturation. The distances the boys lived from the Khimprom factory at the time of the study, along with other demographic characteristics, are presented in Table 1. The mean (sd) distance from the Khimprom factories was 4.45 (1.76) km. The distance from the Khimprom factory to the mother's residence during pregnancy with the index son was self-reported as less than 2 kilometers (8 of 29, or 27%), 2-6 kilometers (8 of 29, or 27%), and greater than 6 kilometers (13 of 29, or 45%). The median duration of residence in Chapaevsk was 15 years.

The concentrations and WHO-TEQ levels for 7 PCDD congeners, 9 PCDF congeners, and 3 non-ortho (coplanar) PCBs are shown in Table 2. The mean (sd) of the sum of PCDDs was 104 pg/g lipids (78.0), and ranged from 8.8 to 318. Only two of the 30 boys had levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) greater than the detection limits; the 30 serum samples weighed 4.1g to 12.1g, and the detection limits for 2,3,7,8-TCDD ranged from 1.5 to 16 pg/g lipids. The 2,3,7,8-TCDD levels for these two boys were 17.9 and 21.7 pg/g. We were unable to identify unique characteristics of these two boys based on the questionnaire data. There was also no difference in dioxin levels between the group of boys with cryptorchidism, hypospadias or delayed puberty and the control boys.

Serum concentrations of PCDDs, PCDFs, and coplanar PCBs accounted for a mean of 34.3% (range 8.4 to 80.1%), 18.0% (range 4.4 to 57.5%), and 47.7% (range 15.5 to 73.9%), respectively of total dioxins. Because of the relatively high concentrations of mono-ortho PCBs they contributed significantly to TEQs and were included in the total TEQs. Based on TEQs, PCDDs, PCDFs, coplanar PCBs, and mono-ortho PCBs accounted for a mean of 11.9% (range 0.14 to 59.0%), 30.4% (range 3.9 to 71.3%), 25% (range 8.1 to 43.0%), and 32.8% (range 0 to 60.1%), respectively, of total TEQs.

In regression models adjusted for age, the log sum of dioxin concentration was associated with increasing age, consumption of locally obtained goat and other non-chicken meat, local meat as a whole (including both chicken, goat and other non-chicken meat), and fish, and log of lipid-adjusted PCB congener 118 (Table 3). For example, a one year increase in age increased the mean sum of dioxins (291 pg/g lipids) by 31%. Gestational age was inversely associated with the sum of the dioxin concentrations with a multiplicative factor of 0.92. Although the coefficient for current residential distance from Khimprom was negative (which supports a decrease in dioxin levels with increasing distance from the factory), it was not statistically significant. The relationship of sum of dioxins and GIS-determined distances from the Khimprom plant was also negative, but not statistically significant. Reproductive history characteristics (gravidity, parity, and prior breastfeeding duration) were also evaluated but not found to be predictive of the sum of dioxins.

Models predicting the log of the sum of dioxin TEQ's were generally similar to the results in Table 3, but suggested weaker effects of age (multiplicative factor=1.28, p=0.20) and local fish consumption (multiplicative factor=1.43, p=0.33), and a stronger effect of log of PCB 118 TEQ. The multiplicative factor estimated for log(PCB 118 TEQ) was 2.74 (95% interval 1.85-4.04).

The best fitting multivariate models are summarized in Table 4. Even after accounting for the strong relationship of PCB 118 with dioxin concentrations, consumption of local goat and other non-chicken meat showed a significant association with dioxin levels. Adjusted R-square values ranged from 0.49 to 0.52. The best fitting model without log PCB 118 included age, gestational age, local goat/other non-chicken meat consumption, local dairy consumption, and income level (<1000 rubles, 1000-1500 rubles, or >1500 rubles per month).

Akhmedkhanov and coworkers[6] recently published data on dioxin levels in twenty-four self-selected volunteers (12 men, mean age 44 years and 12 women, mean age 45 years) living in Chapevsk, Russia, none of whom worked at the Khimprom plant. These adults provided blood samples in 1998 for analysis of dioxins, furans, and PCBs, which were analyzed by the same CDC laboratory that analyzed the boys' serum samples. The mean WHO-TEQ of total dioxins in the 24 adults was 61.2 pg/g lipid (range 16.4 to 168.1 pg/g lipid). The boys and adults from Chapaevsk had the same congeners as major contributors to TEQ of dioxin, furan and coplanar PCBs.

In conclusion, older age and consumption of local foods, including meat and fish, were predictors of the sum of dioxins in serum from adolescent boys in Chapaevsk. There was a suggestive, though non-significant, inverse association between current residential distance to Khimprom factory and sum of dioxins. The lack of an association between reproductive history (gravidity, parity, and prior breastfeeding duration) and sum of dioxins may be due to the older age of the children in the present study (14 to 16 years). In studies in which associations between reproductive history and sum of dioxins are reported, the children are much younger (5-10 years of age) than those in our study. Studies have shown that breast-feeding was an important predictor of early life exposure to 2,3,7,8-TCDD, but that levels in breastfed and non breast-fed children merged at approximately seven years of age[5]. As expected, serum PCBs, specifically PCB 118, were strongly associated with both sum of dioxins and TEQs. The results of our pilot study helped guide the exposure assessment design of a prospective cohort we recently began on 500 eight- and nine- year old boys to determine the relationship between dioxins and pubertal growth and maturation.

Table 1: Demographic characteristics of adolescent boys from Chapaevsk, Russia (N=30)

	Mean (SD)	*Median*	*Minimum*	*Maximum*
Age (years)	15.3 (0.75)	15.2	14.1	16.4
Body Mass Index (kg/m2)	19.9 (3.09)	19.2	15.8	27.7
Weeks of Gestation	39.2 (2.37)	40	30	41
Breastfeeding duration (weeks)	36.9 (25.2)	36	0	96
Birth weight (gm)	3433 (439)	3500	2450	4300
Current distance to Khimprom factory (km)	4.45 (1.76)	4.68	1.53	7.81

Note: 1 subject was missing data on weeks of gestation and breastfeeding, and 2 subjects were missing data on birth weight.

Table 2: Concentrations and WHO-TEQ levels of dibenzodioxin, dibenzofuran, and PCB congeners in blood of 30 adolescent boys from Chapaevsk, Russia

Congener	**# of Samples above MDL**	**Concentration (pg/g lipid)**		**WHO TEF**	**WHO-TEQ (pg TEQ/g lipid)**
		Median	**Mean (SD)**		**Mean (range)**
PCDDs					
2,3,7,8-TCDD	2/29	0	1.37 (5.13)	1.000	1.37 (0.0-21.7)
1,2,3,7,8-PeCDD	12/30	0	3.72 (5.21)	1.000	3.72 (0.0-15.3)
1,2,3,6,7,8-HxCDD	1/30	0	1.71 (9.38)	0.100	0.17 (0.0-5.14)
1,2,3,7,8,9-HxCDD	3/30	0	1.08 (2.66)	0.100	0.11 (0.0-1.05)
1,2,3,4,6,7,8-HpCDD	15/30	14.9	18.1 (11.6)	0.010	0.18 (0.02-0.47)
1,2,3,4,6,7,9-HpCDD	0/30	0.20	1.13 (1.57)	0.0000	0.00 (0.0-0.0)
OCDD	15/29	75.0	79.9 (61.3)	0.0001	0.01 (0.0-0.03)
PCDFs					
2,3,7,8-TCDF	0/30	0	0 (0)	0.100	0.0 (0.0-0.0)
1,2,3,7,8-PeCDF	1/30	0	2.10 (6.24)	0.050	0.11 (0.0-1.46)
2,3,4,7,8-PeCDF	29/30	12.6	20.4 (26.1)	0.500	10.2 (0.0-63.0)
1,2,3,4,7,8-HxCDF	27/30	9.15	24.8 (45.3)	0.100	2.48 (0.34-23.9)
1,2,3,6,7,8-HxCDF	21/30	5.70	8.99 (13.4)	0.100	0.90 (0.00-6.75)
1,2,3,7,8,9-HxCDF	0/30	0	0.013 (0.073)	0.10	0.00 (0.00-0.04)
2,3,4,6,7,8-HxCDF	1/30	0	0.91 (1.33)	0.10	0.09 (0.00-0.51)
1,2,3,4,6,7,8-HpCDF	0/30	5.30	6.53 (4.62)	0.01	0.07 (0.00-0.20)
1,2,3,4,7,8,9-HpCDF	0/30	0	0.02 (0.11)	0.01	0.00 (0.00-0.01)
Coplanar PCBs					
3,4,4',5-TCB81	2/30	0	1.87 (4.47)	0.0001	0.00 (0.00-0.00)
3,3',4,4',5-PeCB126	30/30	68.4	79.4 (40.7)	0.1000	7.94 (2.88-17.2)
3,3',4,4',5,5'-HxCB169	28/30	44.2	41.7 (20.3)	0.0100	0.42 (0.00-0.92)
Total PCDDs		95.8	104 (78.0)		5.51 (0.02-42.1)
Total PCDFs		33.9	63.8 (89.9)		13.9 (0.59-95.2)
Total PCDD/Fs		141	168 (127)		19.4 (0.74-95.5)
Total Coplanar PCBs		120	123 (53.1)		8.35 (3.11-17.9)
Total PCDD/F/coplanar PCBs		273	291 (167)		27.7 (6.57-114)
Total TEQ (including mono-ortho PCBs)					38.5 (12.9-133)

Note: 1,2,3,4,7,8-HxCDD, OCDF, and 3,3',4,4'-TCB 77 were not reported due to interferences in method blanks

Table 3: Individual predictors of log sum of dioxins, adjusted for age in years

Predictor	**Estimate**	**(p-value)**	**Multiplicative factor on dioxin (95% CI)**	**R-Square Value**
Age (years)	0.27	0.065	1.31 (1.00-1.72)	0.12
BMI (kg/m2)	0.03	0.44	1.04 (0.95-1.13)	0.14
Local Meat (y/n)	0.56	0.059	1.75 (1.01-3.04)	0.26
Local Goat/Other Meat (y/n)	0.58	0.032	1.78 (1.08-2.93)	0.29
Local Chicken (y/n)	0.14	0.51	1.15 (0.77-1.73)	0.16
Local Fish (y/n)	0.48	0.077	1.62 (0.97-2.69)	0.24
Local Eggs (y/n)	0.22	0.32	1.25 (0.82-1.90)	0.18
Local Dairy (y/n)	-0.12	0.77	0.89 (0.39-2.00)	0.15
Local Fruits/ Vegetables (y/n)	-0.09	0.88	0.91 (0.30-2.81)	0.15
Distance from Khimprom (km)	-0.07	0.25	0.93 (0.82-1.05)	0.16
Weeks of Gestation (weeks)	-0.08	0.077	0.92 (0.85-1.01)	0.24
Weeks of breast-feeding (per 12 weeks)	-0.003	0.96	1.00 (0.90-1.10)	0.14
log (PCB 118) (ng/g lipid)	0.66	0.001	1.94 (1.38-2.72)	0.44
Income level (low, medium, high)	-0.10	0.44	0.90 (0.70-1.16)	0.16

Note: All models are adjusted for age in years.

N=30 for age, BMI, and distance from Khimprom at time of blood draw; N=29 for all food consumption and medical history predictors; N=27 for model with PCB118.

Table 4: Best fit multivariate models for predicting log sum of dioxin concentrations among boys in Chapaevsk, Russia, including and excluding PCB118 as a predictor

Model #	Predictor	Estimate	p-value	R-square value	Adjusted R-square value
1	Local Goat/Other Meat (y/n)	0.59	0.017	0.56	0.52
	log (PCB 118) (ng/g lipid)	0.63	<0.001		
2	Local Goat/Other Meat (y/n)	0.94	<0.001	0.58	0.49
	Age (years)	0.44	<0.001		
	Weeks of Gestation	-0.11	0.004		
	Income Level (low,med,high)	-0.28	0.013		
	Local Dairy (y/n)	-0.67	0.053		

N=26 for model 1; N=29 for model 2. Missing data for 3 boys on lipid-adjusted PCB118, and one boy on questionnaire items (medical history, diet). Model 1: best fit multivariate model (backwards selection and Mallows C(p)) including PCB118 data. Model 2: best fit multivariate model (forward and backward selection) not including PCB118 data.

Acknowledgement: This work was funded by the US EPA, Grant number R-82943701-0

References

1. Revich B., Aksel E., Dvoirin V., Kolbeneva L.and Pervunina R. (1996) Organohalogen Compounds. 30: 350-353.
2. Revich B., Aksel E., Ushakova T., Ivanova I., Zhuchenko N., Kluev N., Brodsky B. and Sotskov Y. (2001). Chemosphere 43:951-966.
3. Lee MM, Sergeyev O, Williams P, Korrick S, Zeilert V, Revich B and Hauser R. (2003). J Pediatr Endocrinol Metabol 16:169-178
4. Turner W., DiPietro E., Lapeza C., Green V., Gill J. and Patterson, D.G. , Jr.(1997). Organohalogen Compounds 31, 26-31.
5. Kreuzer PE, Csanady GA, Baur C, Kessler W, Papke O, Grein H and Filser JG. (1997) Arch Toxicol 71:383-400.
6. Akhmedkhanov A., Revich B., Adibi J.J., Zeilert V., Masten S.A., Patterson D.G. Jr., Needham L.L. and Toniolo P. (2002). Journal of Exposure Analysis and Environmental Epidemiology 12:409-417.