Dioxins and Pubertal Development Among Boys in Chapaevsk, Russia

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Specific Aims

- **Specific Aim #1:** To investigate the relationship between exposure to dioxin-like compounds and non dioxin-like PCBs with the timing and tempo of pubertal development.
  - Specifically interested in relationships with age of pubertal onset, alterations in tempo of pubertal progression, and age at attainment of sexual maturation.

Specific Aims

- **Specific Aim #2:** To investigate the relationship between exposure to dioxin-like compounds and non dioxin-like PCBs with linear growth, weight gain, and body mass index (BMI).
  - These alterations in somatic growth may secondarily promote earlier pubertal maturation.

Specific Aims

- **Specific Aim #3:** To investigate the relationship between serum levels of dioxin-like compounds and non dioxin-like PCBs with biochemical changes in hormones that regulate growth and pubertal maturation.
  - Specifically interested in alterations in concentrations of sex steroids (testosterone), peptide hormones (inhibin B and Müllerian inhibiting substance (MIS)), gonadotropins (luteinizing and follicle stimulating hormones) and triiodothyronine (T_3) and thyroxine (T_4).

Background: Epidemiologic Studies

- Growth and pubertal development in boys.
  - North Carolina Cohort: In utero exposure to DDE associated with increased height and weight adjusted for height. No relationship of DDE with pubertal maturation. PCBs were not related to growth or pubertal maturation.
  - Belgium Study: PCBs were associated with delayed pubertal maturation in boys from a polluted area compared with a rural area. Decreased testicular volume in boys from the polluted area. Used the Calux assay to measure biologically active PCAHs.

- Note: Several studies in girls on PBBs, PCBs, DDE and age of menarche and pubertal development.

Background: Toxicological Studies

- Dioxins are developmental and reproductive toxicants (especially for the developing male).
    - Stage at which exposure occurs affects sensitivity (in utero > pubertal > adult)
    - Evidence of testicular toxicity
        - Pubertal exposure alters spermatogenesis and sperm function (motility).
    - Androgenic deficiency, decreased estrogen and altered regulation of LH secretion.
    - Alterations of key pathways involved in signal transduction of growth factors and sex steroid hormones involved in the control of gonadal development.

Project Timeline

- **1999:** Pilot study on 2580 boys, age 10-16 years, to generate normative data for height and weight, and data on the distribution of Tanner stages by age
  (Lee et al. J Ped Endo Meta 2003)

- **2000:** Among boys in the pilot study, blood samples were drawn from a subset of 221 boys 14-16 yrs of age.
  - Measured dioxins, furans and PCBs to explore associations with diet, reproductive history, residential distance to factories, etc. (Hauser et al. Environ Health 2005)

- **2002:** U.S.E.P.A. funding for prospective cohort study on adolescent boys. Recruitment for the prospective cohort study began in 2003 and was completed in May 2005.

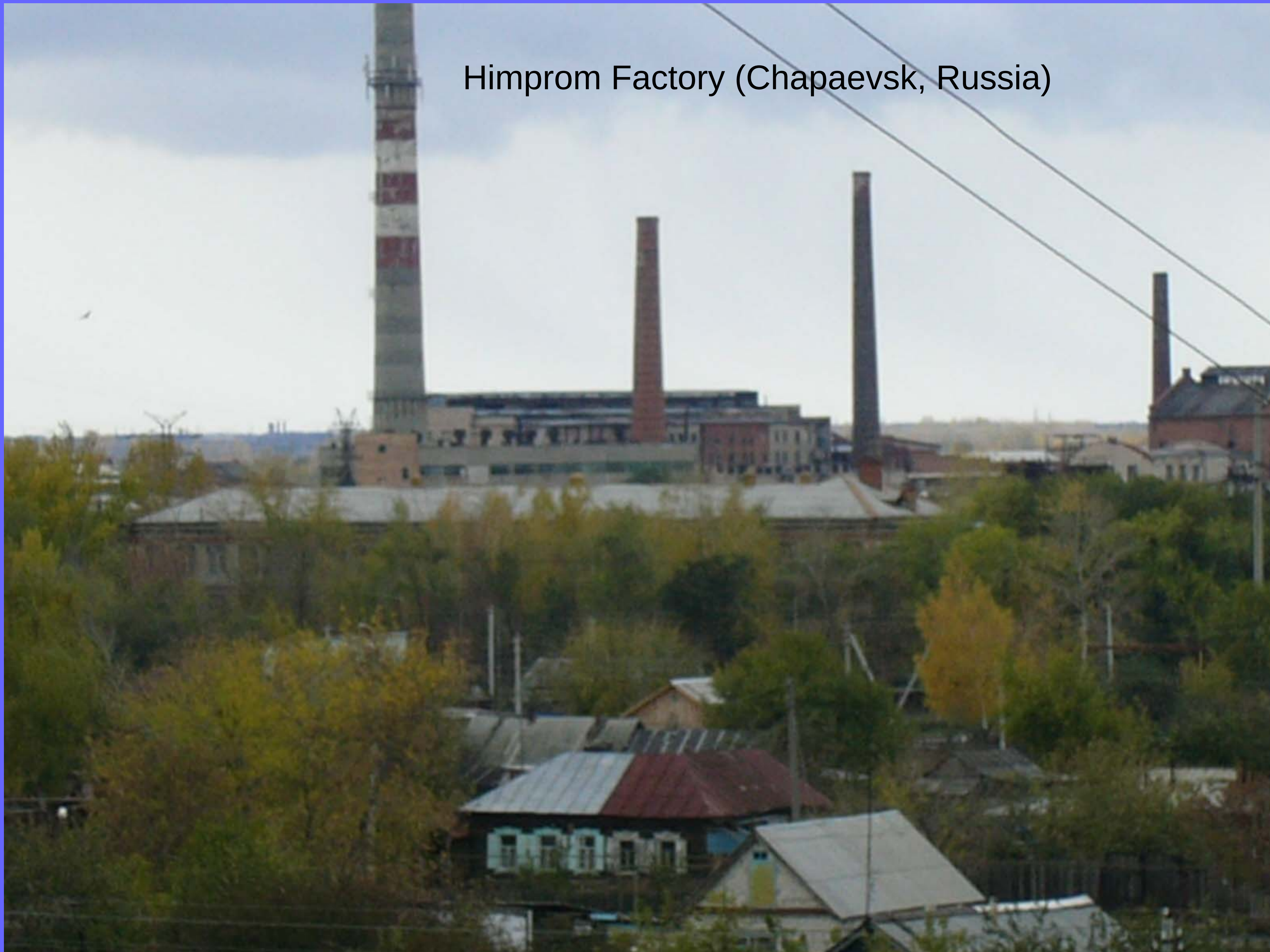
Study Site

- Chapaevsk is a small industrial city in Russia
- It is located 43 km southwest of Samara, on the Chapaevka River, which flows into Volga River
- Population: 83,000; area=187 km^2
- Half of the city is occupied by military or chemical industries
- Middle Volga Chemical plant (Himprom) – produced chemical weapons and since 1967 (to 1987) produced hexachloro cyclohexane (lindane) and derivatives. Since this time has since produced agricultural pesticides containing chlorine; byproducts of manufacturing process include dioxins and furans.

Himprom factory and surrounding residences (Chapaevsk, Russia)

Himprom Factory (Chapaevsk, Russia)

Himprom Factory (Chapaevsk, Russia)



Progress on Prospective Cohort Study

- Recruitment began in 2003
  - There were delays in setting up a local IRB and shipping supplies to Russia (the paperwork and customs approval process were very very difficult!)
- To date:
  - 516 boys and their mother's have been recruited.
  - Baseline visits:
    - Physical examinations, blood and urine samples (mother and son), questionnaires.
  - Annual visits:
    - Physical examinations, blood (every two years) and urine samples (son only), questionnaire updates.

Study Methods

- Questionnaires
  - Medical history, lifestyle, and dietary information (consumption of local foods, as well as food frequency questions)

- Anthropometric Measurements
  - Height and weight ; Skin fold thickness (calipers)
  - Bioelectric impedance (added in 2006)

- Physical Maturation
  - Testicular volume (Prader’s orchidometer)
  - Tanner stages (genital and pubic)

- Physiological Measure
  - Hormones (LH, FSH, T, Inhibin-B, SHBG, Pr, MIS, TSH, T_3, T_4)

- Biological Specimens for Exposure Assessment
  - Serum samples collected to measure dioxin/furan/PCBs
  - Urine samples archived

Recruitment visit (Chapaevsk, Russia)

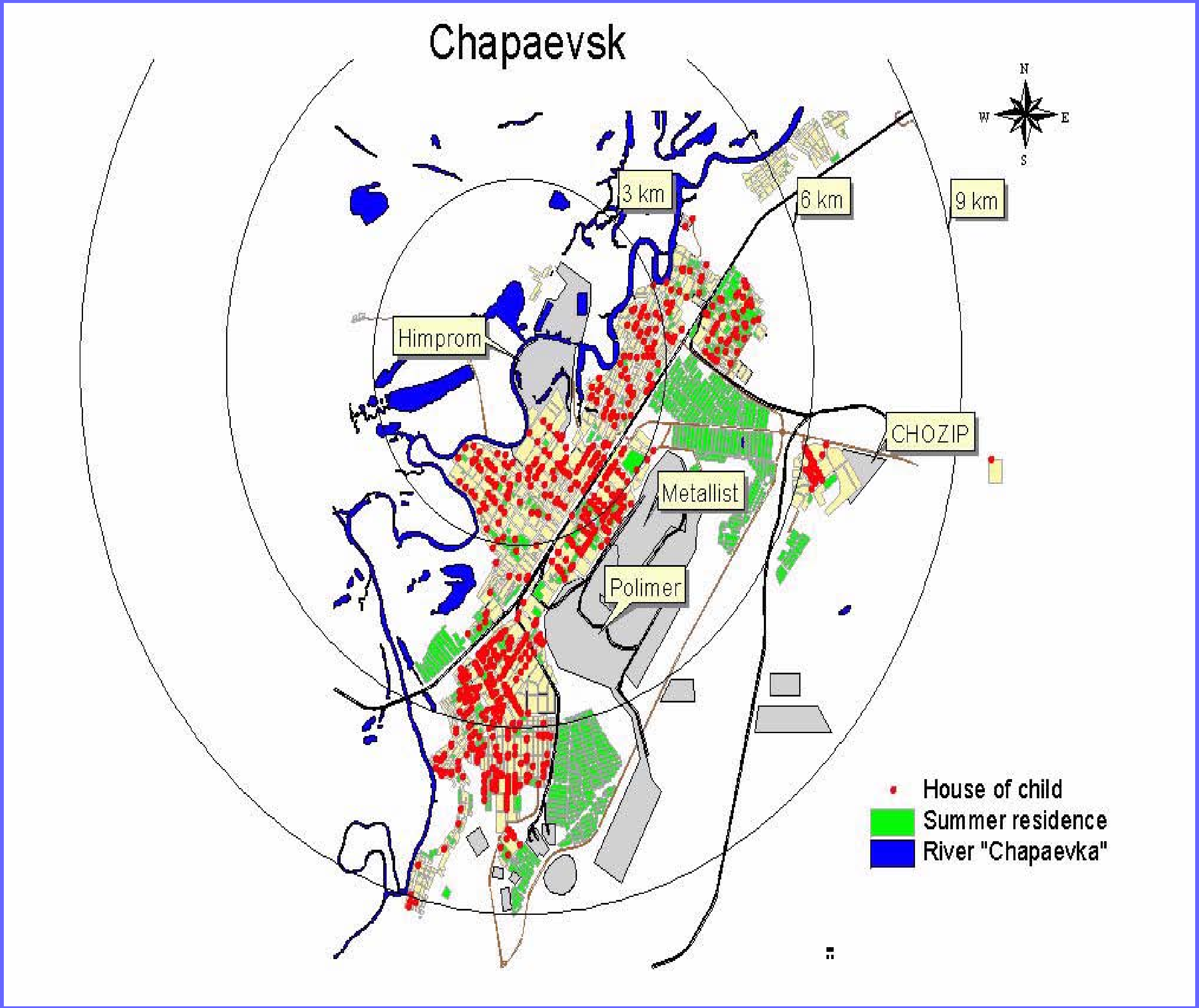

Не сжигай отходы - это источник диоксинов
Старайся уменьшить количество отходов
PVC
Откажись от использования хлорсодержащих пластмасс
DFID

DATA

GIS Mapping: 1999 Pilot Study Residential Addresses

- Generated maps of the distribution of participants' residential location relative to the Himprom factory and other factories.
- The electronic map of Chapaevsk was constructed with the use of a geographic map of scale 1:10000 and ArcView GIS 3.0.
- The dots on the map approximate the residential addresses of the boys and were placed using a geocoding process.

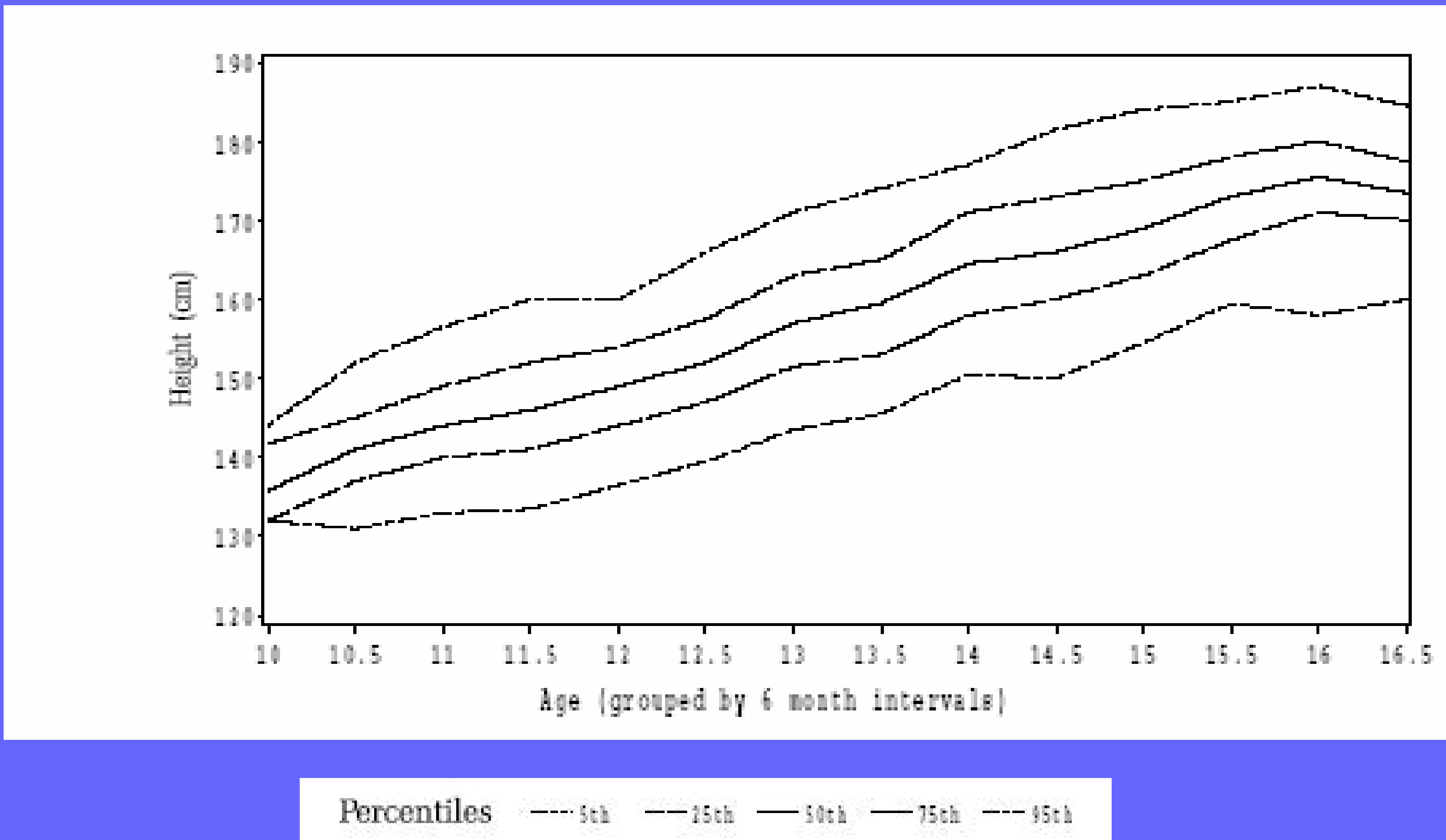
Figure 1: Distance from the Himprom factory for the 2580 boys enrolled in phase one of study



Sergeyev et al. (Dioxin 2002)

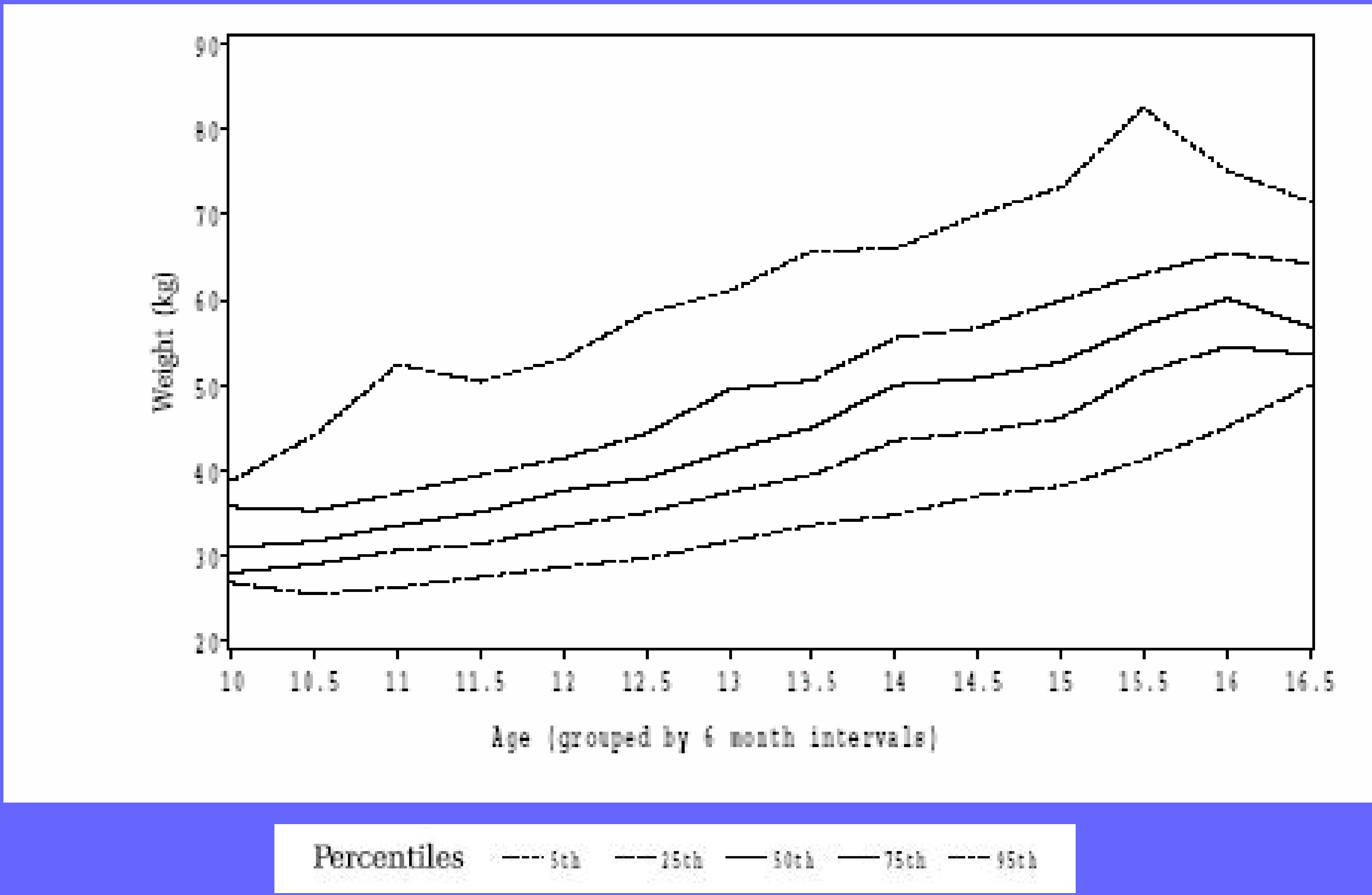
Growth curves from Pilot Study

Figure 2a: Percentiles of Height by Age Group



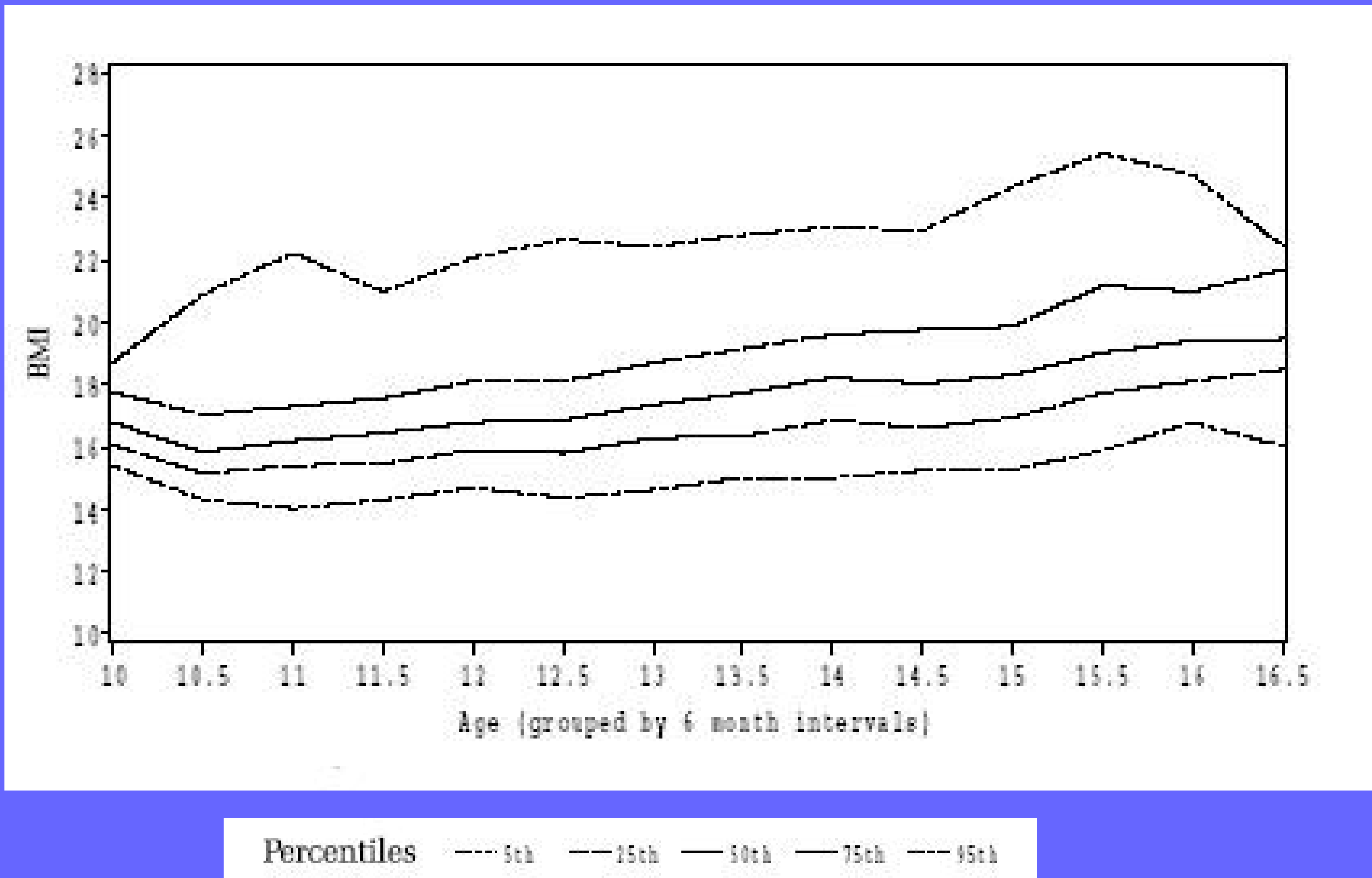
Lee et al. (J Ped Endo Metab 2003)

Figure 2b: Percentiles of Weight by Age Group



Lee et al. (J Ped Endo Metab 2003)

Figure 2c: Percentiles of Body Mass Index (BMI) by Age Group



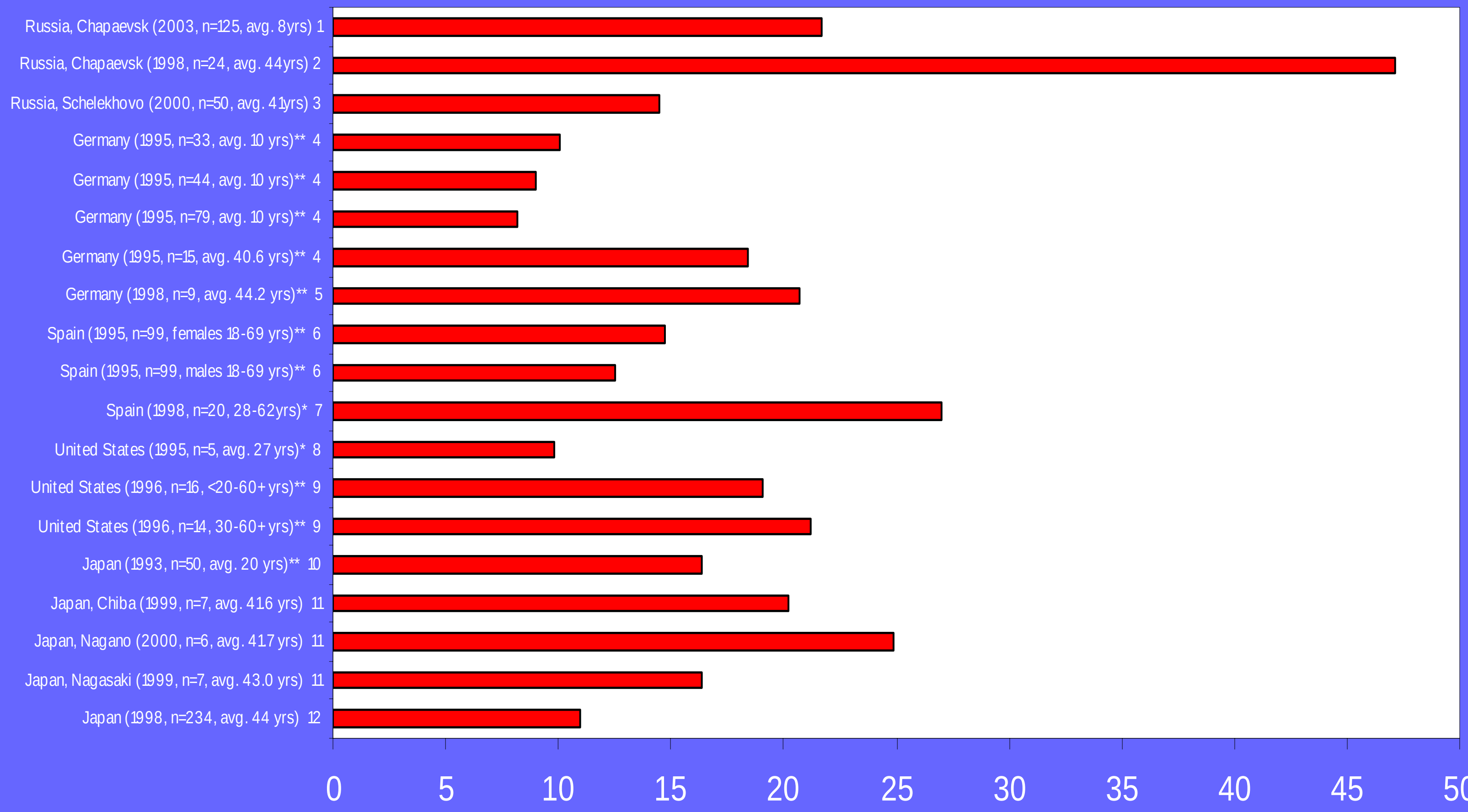
Lee et al 2003 (J Ped Endo Metab 2003)

Distribution of and Predictors of Dioxin Exposure

Table 1: Distribution of PCDDs/PCDFs/PCBs in blood of 125 adolescent boys in Chapaevsk, Russia

	Concentration (pg/g lipid) Median (25^{th}, 75^{th})	WHO-TEQ (pg TEQ/g lipid) Median (25^{th}, 75^{th})
Total PCDDs	160 (124, 221)	9.8 (5.3, 14.3)
Total PCDFs	40.9 (27.5, 58.4)	7.3 (4.6, 10.8)
Total Co-PCBs	290 (243, 366)	8.4 (6.2, 13.0)
Total PCDD/F/ Co-PCBs	497 (431, 653)	25.1 (17.2, 39.1)
Total TEQs		39.5 (24.9, 60.2)

Figure 3. Mean PCDD/PCDFs TEQ levels in Chapaevsk boys in comparison with other populations

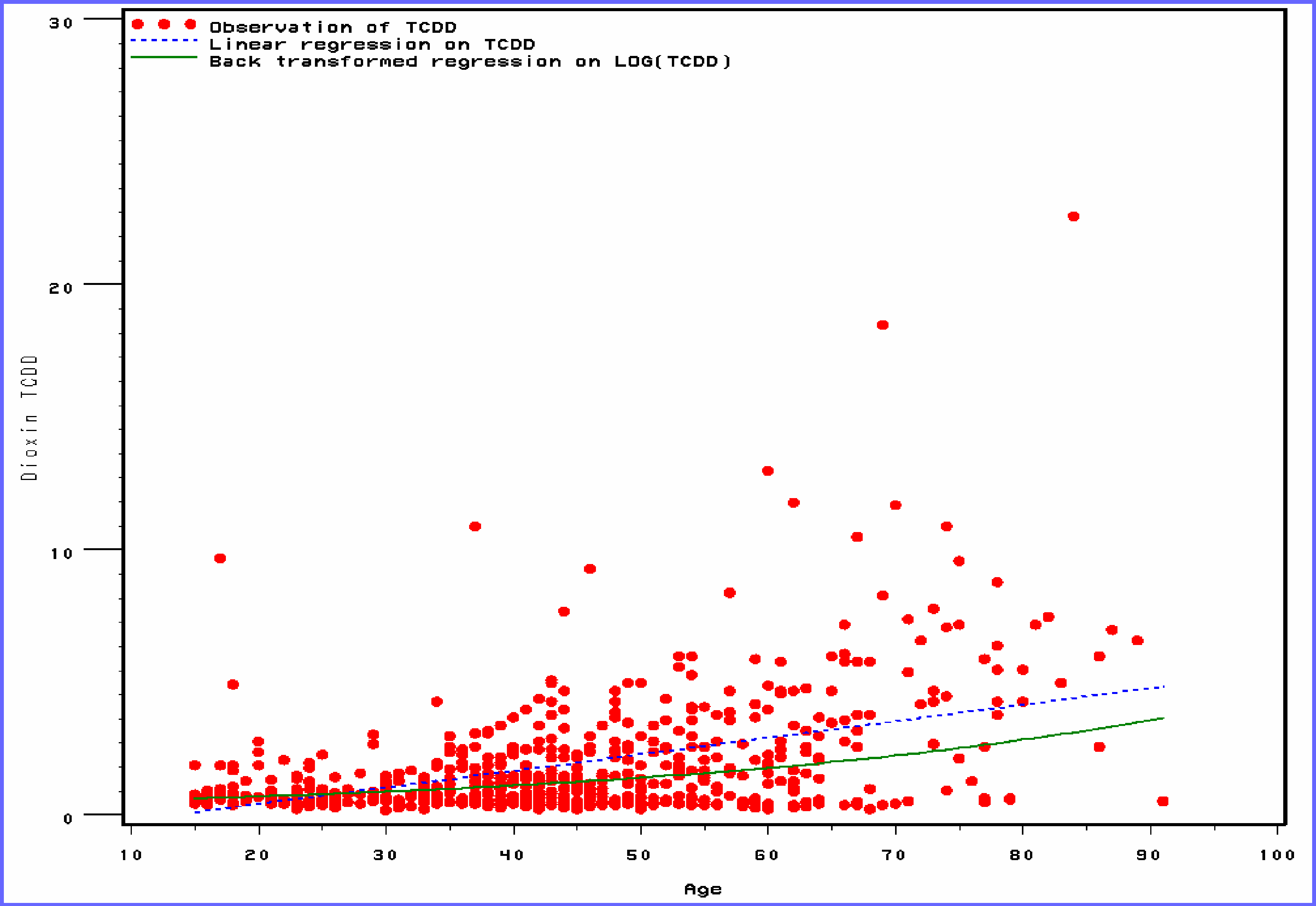


PCDD/F levels are in pgTEQ/g lipid (TEQ-WHO, 1998) unless specified as: * = TEQ-WHO (1994) ** = I-TEQ (1989)

Levels are means except for Tepper (#9) which presented medians

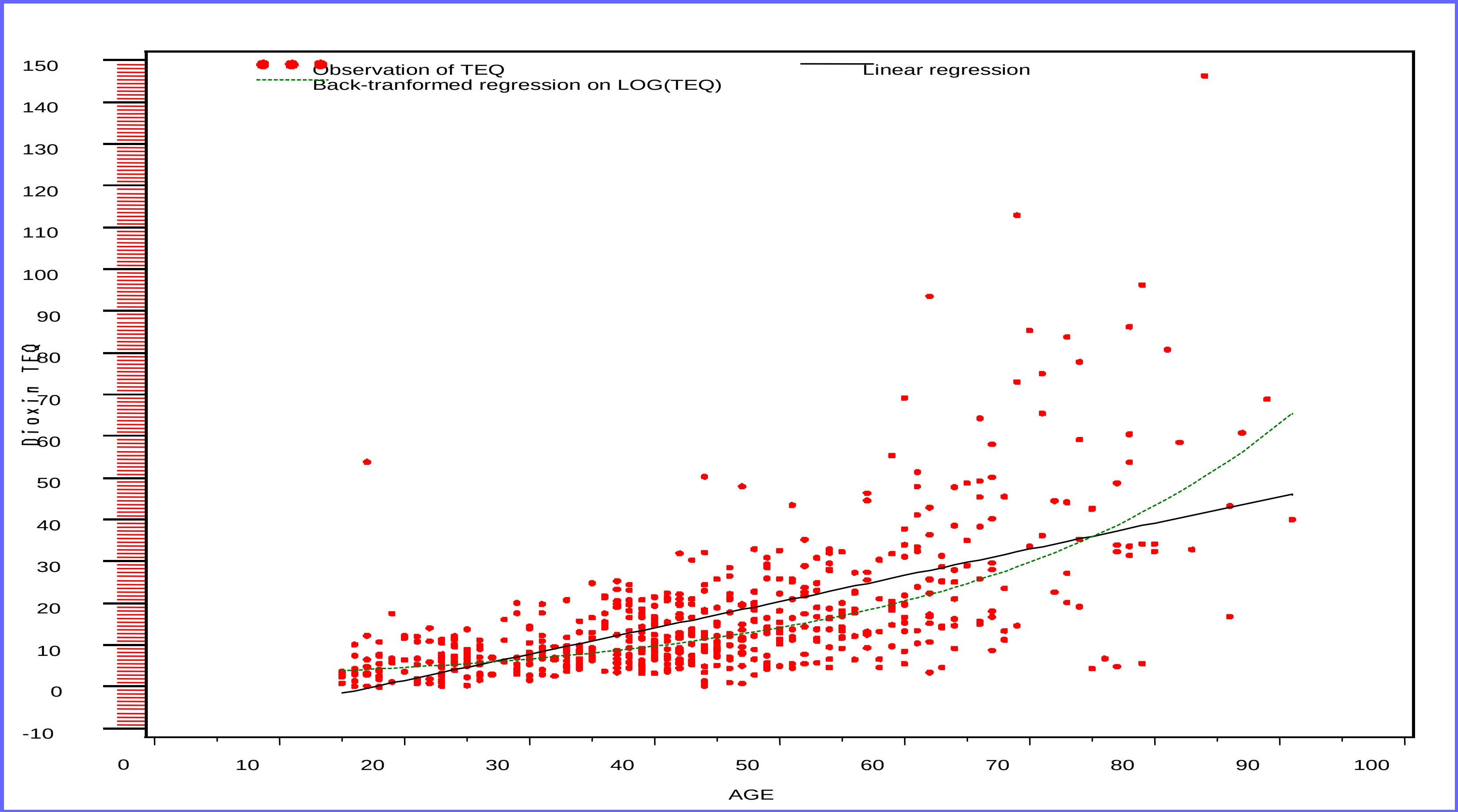
1 Hauser et al - current study
2 Akhmedkhanov et al - 2002
3 Mamontova et al - 2002
4 Wuthe et al - 1996
5 Wittsiepe et al - 2000
6 Gonzalez et al - 1998
7 Schumacher et al - 1999
8 Shechter et al - 1998
9 Tepper et al - 1997
10 Iida et al - 1999
11 Kumagai et al - 2002
12 Ueda et al - 1999

Figure 4: Non-Linear Relationship Between TCDD and Age



Patterson et al. (Dioxin2004)

Figure 5: Non-linear relationship between TEQ and Age



- Linear Regression (P < 0.0001, $R^2 = 0.39$)
  - Significant Correlation is Indicated
- Clearly Not Linear/Range of TEQ Increases with Age

Patterson et al. (Dioxin2004)

Predictors of Dioxin Exposure

- 30 boys (age 14-16 years) from the 1999 pilot study had serum samples analyzed for PCDDs, PCDFs, PCBs (coplanar and ortho substituted).
- Predictors of interest:
  - Age, BMI, Dietary intake, Distance to Himpron factory at time of blood draw and during pregnancy, Duration of residence in Chapaevsk, Birth weight, Weeks of gestation, Weeks of breast-feeding, Parity, Parental education, Income.

Table 2: Predictors of log sum of dioxins* in 30 adolescents from Chapaevsk, Russia

Predictor	Estimate	p-value	Multiplicative factor on dioxin (95% CI)
Age (years)	0.26	0.057	**1.30 (1.00-1.72)**
Local non-chicken meat (y/n)	0.56	0.042	**1.75 (1.05-2.92)**
Local Fish (y/n)	0.48	0.079	**1.62 (0.97-2.71)**
Distance from Khimprom (km)	-0.06	0.37	**0.94 (0.82-1.07)**
Weeks of Gestation	-0.08	0.072	**0.92 (0.84-1.00)**
log (PCB 118) (ng/g lipid)	0.64	<0.001	**1.90 (1.37-2.63)**

Note: All models are adjusted for age in years.

* Sum of dioxin concentrations includes PCDDs, PCDFs, and coplanar PCBs.

Table 3: Best fit multivariate models for predicting log sum of dioxin* concentrations among 30 adolescent boys in Chapaevsk, Russia

Model #	Predictor	Estimate	p-value	R-square value	Adjusted R-square value
1	Local non-chicken Meat (y/n)	**0.59**	**0.017**	0.56	0.52
	log (PCB 118) (ng/g lipid)	**0.63**	**<0.001**		
2	Age (years)	**0.40**	**<0.001**	0.56	0.46
	Weeks of Gestation	**-0.11**	**0.005**		
	Local Dairy	**-0.76**	**0.037**		
	Local non-chicken Meat (y/n)	**0.90**	**<0.001**		
	Income Level (low, med, high)	**-0.27**	**0.020**		

* Sum of dioxins includes PCDDs, PCDFs, and coplanar PCBs; Model 1: best fit multivariate model including PCB 118 data; Model 2: best fit multivariate model (forward and backward selection) not including PCB 118.

Summary of Progress

- Generated Normative growth curves for height, weight, and BMI among Chapaevsk boys 10 to 17 years old

- Confirmed wide distribution of dioxin levels among boys in Chapaevsk

- Identified predictors of dioxin levels (included dietary measures, reproductive history and residential location)

- Assembled a cohort of pre-pubertal boys for longitudinal study
  - Annual follow-up with participation over 90%
  - Yearly physical examination and questionnaire updates
  - Biannual blood samples

- Obtained NIEHS funding to continue follow-up (5-yrs)

Future Plans

- Analyze boy’s and mother’s serum samples for dioxins (in collaboration with CDC)
  - Boy’s exposure at recruitment (pre-pubertal)
  - Estimate boy’s gestational exposure using their current dioxin levels, mother’s current dioxin levels, and information from reproductive history of mother (number of children, breast feeding history, etc)
- Explore the relationship between pubertal exposure to dioxins and intermediate measures of reproductive function in the children when they reach adulthood (age 18 years). Testicular function will be assessed by semen evaluation and reproductive hormones.

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Questions?