STUDIES IN HUMANS: RESULTS FROM THE RUSSIAN CHILDREN'S STUDY AND OTHER COHORTS

CONTAM Opinion on dioxins and DL-PCBs in food and feed

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CONTAM Panel Chair (2015-2018)

Info Session - 13 November 2018

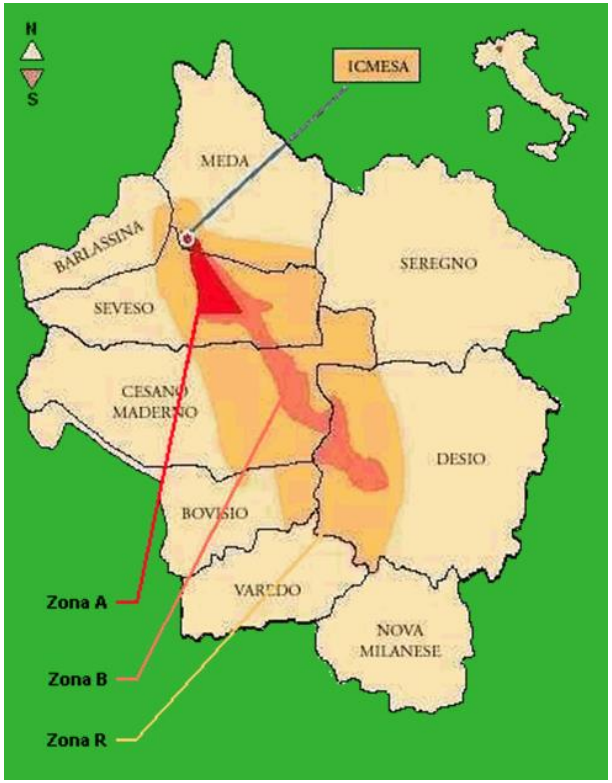
efsa
European Food Safety Authority

Outline

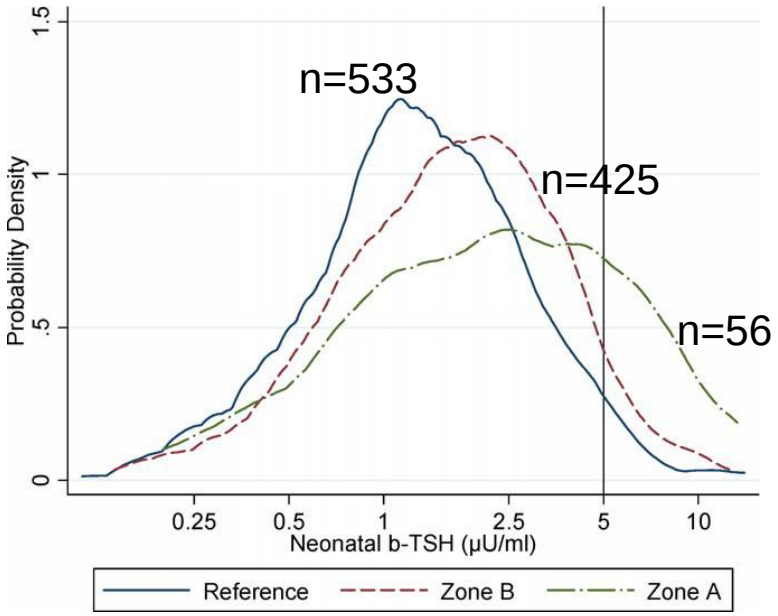
- Outcomes in humans – other than semen quality
- Semen quality
  - Seveso cohorts
  - Russian Children's Study (RCS)
  - Factors for consideration:
    - ✓ Organochlorine pesticides
    - ✓ Pubertal development
    - ✓ Loss to follow-up
    - ✓ Lead
    - ✓ Alcohol and tobacco consumption
    - ✓ Variability in semen analyses
  - Dose-response assessment
  - Causality of decreased sperm concentration
- Relevance of impaired semen quality

Increased TSH in newborns (maternal exposure)

- Low-moderate exposure/background: no adverse effects
- **Seveso: Residence-based study** (born 1994-2005, n= 1014)
- **Relatively strong support for a causal association**

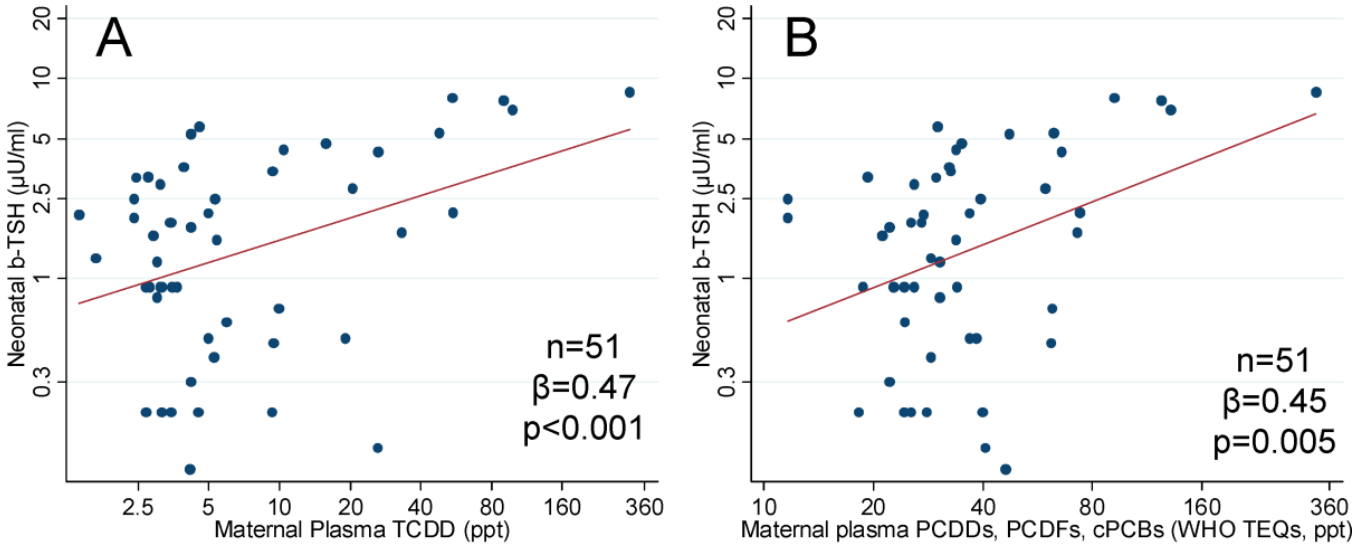


(Baccarelli et al. 2008)



Increased TSH in newborns, Seveso

- Levels in blood from women, collected (1992-1998), 16 to 22 years after the incident (Baccarelli et al. 2008)
  - Higher TCDD levels (>10 pg/g) extrapolated to time of birth
  - TSH in newborn babies (n=51)
  - Association driven by few subjects with high TCDD levels
- *Additional information*: timing of blood TSH missing for several children whose mothers had high TCDD
- Over the first 2-3 days of life TSH decreases factor 5-10
- Not used as basis for the risk assessment

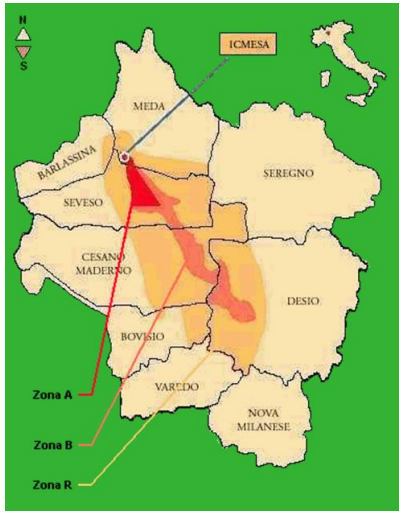


Developmental effects on teeth

- Likely a **postnatal effect**: First permanent molars are mineralized at 0-2 years age, other molars later

- **Seveso 20 years after the 1976 incident**: Prevalence of tooth enamel defects higher in Zone ABR than in non-ABR, odds ratio 2.4 (1.3-4.5) (Alaluusua et al. 2004)

  - Dose-related in zone ABR subjects
  - Primarily in those below 5 years in 1976



Developmental effects on teeth (cont')

- Breast-fed children in Finland, age 6-7, **born 1987**: **Enamel hypo-mineralization** in first permanent molars (Alaluusua et al. 1996, 1999)
  - **Dose-related to total exposure**: milk level **and** duration of breastfeeding
  - Children **born 1995-1999**: no effects (lower level in milk, shorter breastfeeding)

- **Yucheng** incident, Taiwan: Teeth defects higher in children of exposed mothers than controls (Rogan, 1988)

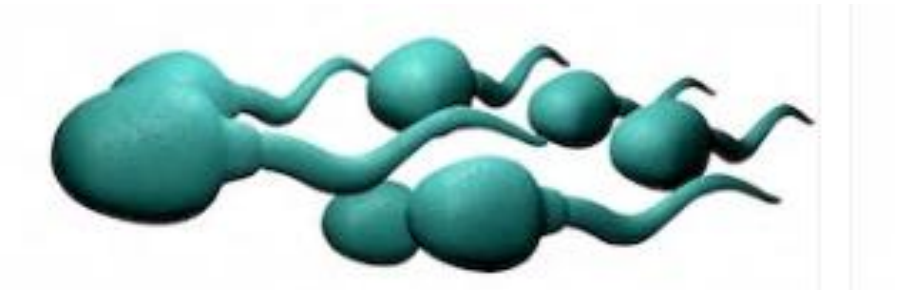
Birth outcome – sex ratio

- **Lower sex ratio** at birth (boys:girls) after paternal exposure:
  - Reported from 3 cohorts (Seveso, Ufa, New Zealand)
  - Appears to be a high-dose effect
  - High uncertainty in the calculation of paternal serum concentration at fertilization

Semen quality

- **CRITICAL EFFECT: Reduced semen quality** (sperm concentration)

- **Evidence from:**
  - Two Seveso cohorts (TCDD)
  - Russian Children's Study (background exposure)



Mocarelli et al. 2008

Dioxin Exposure, from Infancy through Puberty, Produces Endocrine Disruption and Affects Human Semen Quality

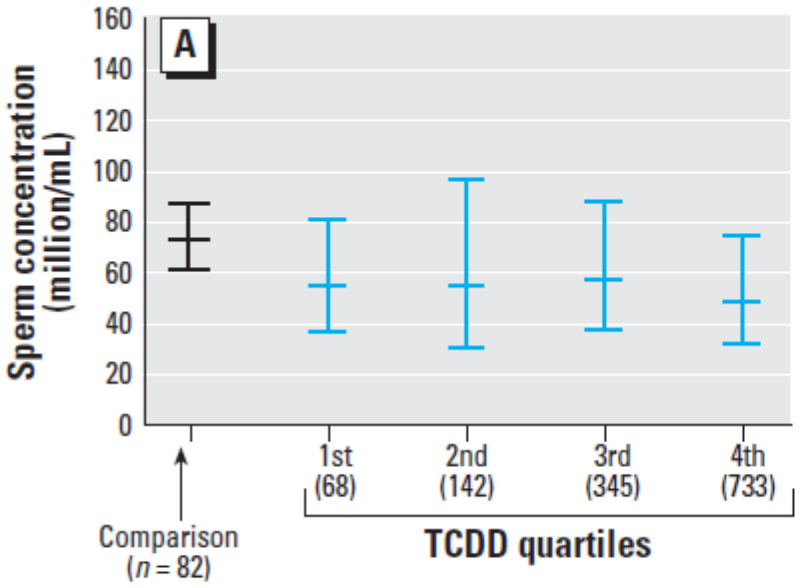
Paolo Mocarelli,[1,2] Pier Mario Gerthoux,[1] Donald G. Patterson Jr.,[3] Silvano Milani,[4] Giuseppe Limonta,[1] Maria Bertona,[1] Stefano Signorini,[1] Pierluigi Tramacere,[1] Laura Colombo,[1] Carla Crespi,[1] Paolo Brambilla,[1] Cecilia Sarto,[1] Vittorio Carreri,[5] Eric J. Sampson,[3] Wayman E. Turner,[3] and Larry L. Needham[3]

VOLUME 116 | NUMBER 1 | January 2008 • Environmental Health Perspectives

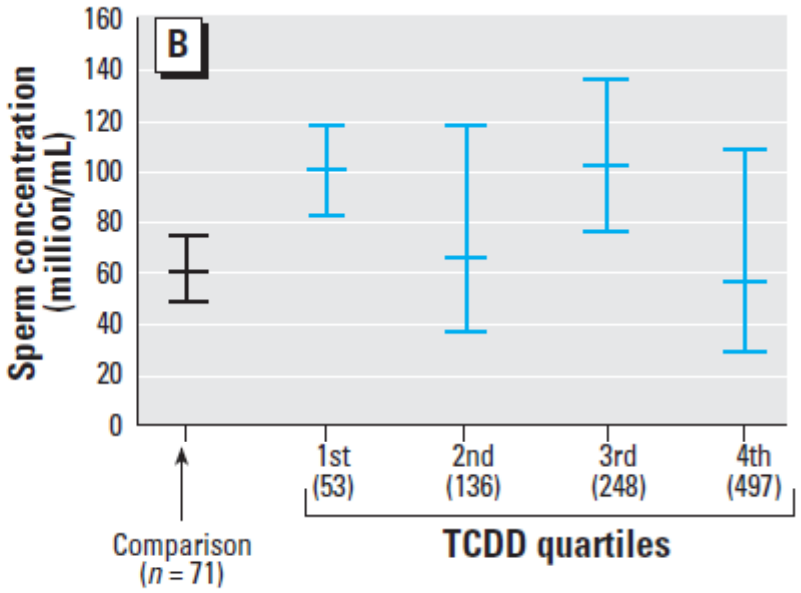
- TCDD measured in blood collected within 1 year after accident
  - No other PCDD/Fs and DL-PCBs measured
- Semen sampled 22 years later
- Compared to controls (blood donors)

Mocarelli et al. 2008 (cont')

1-9 years at accident



10-17 years at accident



≤ 15 pg/g fat
(assumed, not analysed)

- Significant decrease seen only in boys 1-9 years at accident (n=71) versus controls (n=82)
- No dose-response across quartiles
- LOAEL of 68 pg TCDD/g fat (lowest quartile)

Mocarelli et al. 2011

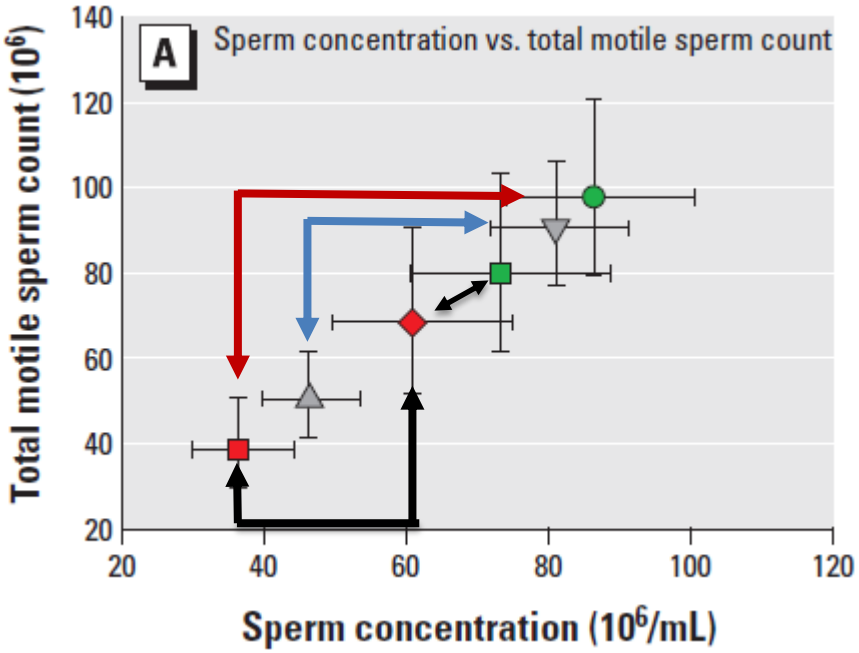
Perinatal Exposure to Low Doses of Dioxin Can Permanently Impair Human Semen Quality

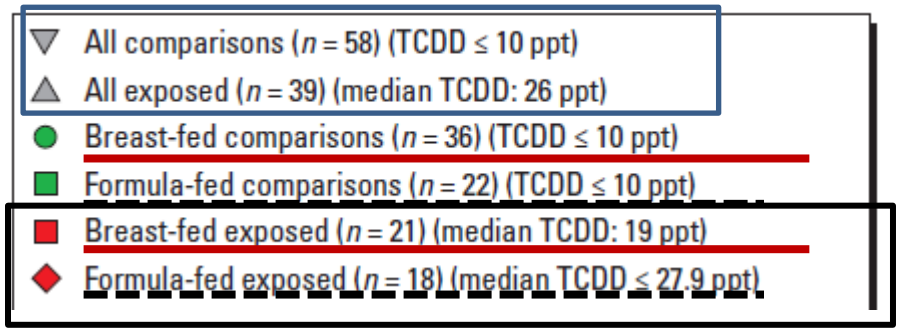
Paolo Mocarelli,[1,2] Pier Mario Gerthoux,[1] Larry L. Needham,[3] Donald G. Patterson Jr.,[3,4] Giuseppe Limonta,[1] Rosanna Falbo,[1] Stefano Signorini,[1] Maria Bertona,[1] Carla Crespi,[1] Cecilia Sarto,[1] Paul K. Scott,[5] Wayman E. Turner,[3] and Paolo Brambilla[1,2]

Environmental Health Perspectives • VOLUME 119 | NUMBER 5 | May 2011

- Effects in men (n=39) exposed via Seveso mothers
  - Born 1977-1984
  - 18 breast-fed, 21 formula-fed
  - Comparisons: 58 blood donors not affected by incident
  - Semen collected at age 18-26 years
- Maternal blood sampled shortly after incident
  - Only TCDD (extrapolated to conception)

Mocarelli et al. 2011 (cont')





- Significant difference all exposed vs all comparison
- Significant difference breast-fed exposed vs breast-fed comparison
- No significant difference formula fed exposed vs comparison
- Not significant p=0.07 Breast fed exp vs formula fed exposed
- **Indicates a postnatal effect on sperm concentration**

CRITICAL STUDY

Russian Children's Study

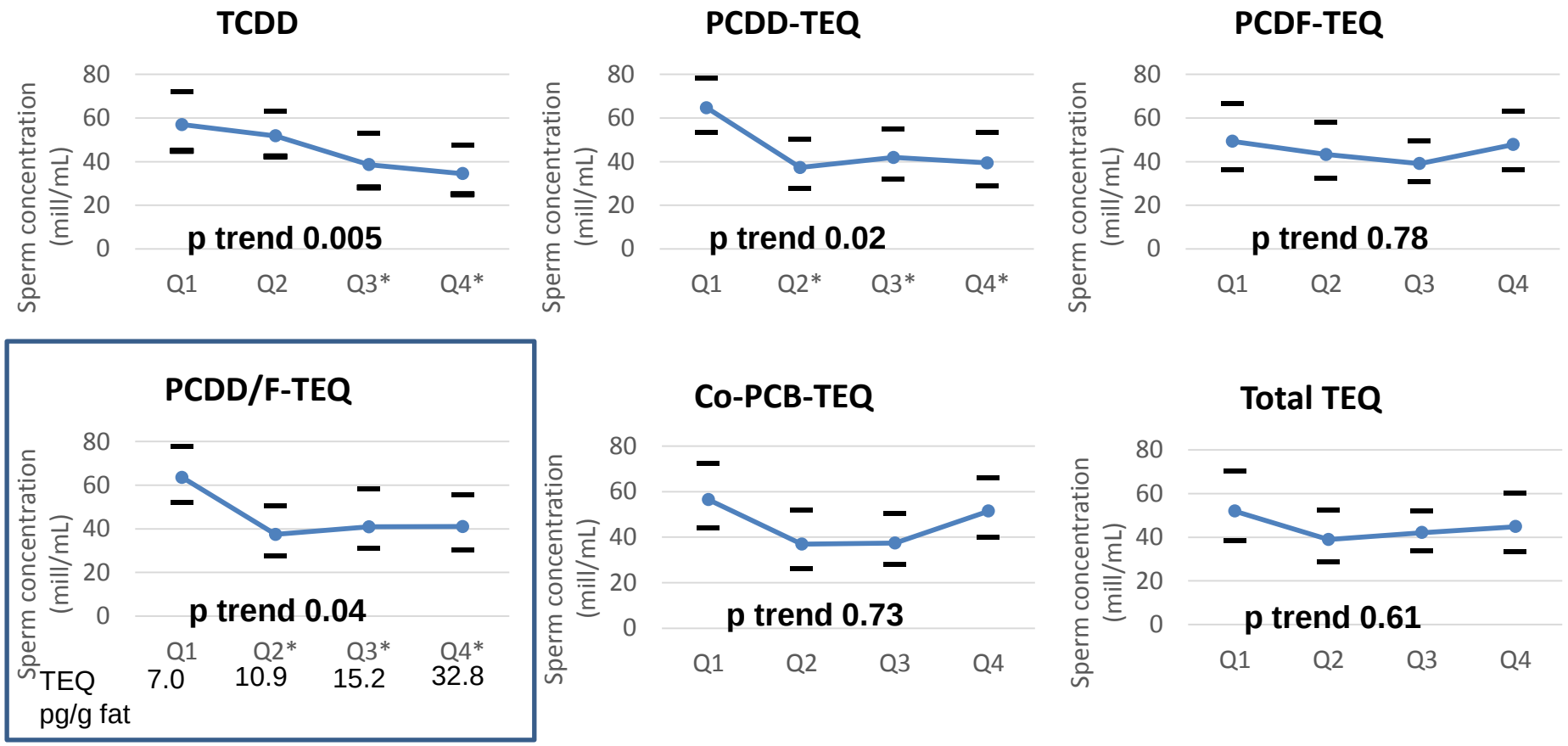
A Longitudinal Study of Peripubertal Serum Organochlorine Concentrations and Semen Parameters in Young Men: The Russian Children's Study

Lidia Mínguez-Alarcón,[1] Oleg Sergeyev,[2,3] Jane S. Burns,[1] Paige L. Williams,[4,5] Mary M. Lee,[6] Susan A. Korrick,[1,7] Luidmila Smigulina,[3] Boris Revich,[8] and Russ Hauser[1,5]

VOLUME 125 | NUMBER 3 | March 2017 · Environmental Health Perspectives

- Chapaevsk (Russia): former production of chlorinated pesticides (ceased 1987)
- Boys born in 1994-95
  - Short-lived substances less relevant
- Background exposure (food)
- Blood sampled at age 8-9 years (2003-2005)
  - PCDD/Fs and DL-PCBs, NDL-PCBs, OCPs, lead
- Semen sampled at age 18-19 (n=133, 256 semen samples)

Sperm concentration (Mínguez-Alarcón et al. 2017)



Multivariable adjusted mean (95% CI) sperm concentrations by quartiles of serum levels at age 9 years among 133 men (256 semen samples) at age 18-19 years in the Russian Children's Study.

Adjustment: BMI, smoking status, alcohol drinker, season, abstinence time

Figure based on data in Mínguez-Alarcón et al. (2017) and data submitted to EFSA.

Opinion Table 10 updated (error in quantile TCDD)

Table 10. Multivariable adjusted mean semen parameters by quartiles of serum PCDDs, PCDFs and PCBs among 133 young men in the Russian Children's Study contributing 256 semen samples. The table is based on data in Mínguez-Alarcón et al. (2017) and data submitted to EFSA by the study authors (*in italics*) (see Documentation provided to EFSA).

	Median	Volume (mL)	Sperm Concentration (mill/mL)	Total Sperm Count (mill)	Motile Sperm (%)	Total Motile Sperm Count (mill)
TCDD [(a)] (pg/g fat)						
Q1 [0.35-1.70]	0.77	2.7 (2.2, 3.2)	57.0 (45.0, 72.1)	128 (95.6, 173)	61.6 (58.6, 64.7)	78.0 (56.0, 109)
Q2 [1.77-2.90]	2.45	2.9 (2.5, 3.4)	51.8 (42.4, 63.3)	136 (105.0, 175)	65.4 (63.4, 67.4)	87.9 (67.1, 115)
Q3 [3.00-4.30]	3.40	2.6 (2.1, 2.9)	38.6 (28.2, 52.9)*	85.8 (60.4, 122)	59.5 (56.0, 62.9)	50.1 (33.5, 74.8)
Q4 [4.40-12.1]	5.80	3.1 (2.5, 3.7)	34.5 (25.0, 47.7)*	91.6 (63.5, 132)	60.1 (56.6, 63.7)	54.1 (36.0, 81.4)
p, trend		0.55	0.005	0.05	0.17	0.05
TCDD [(a, b)] (pg/g fat)						
Q1 [0.35-1.70]	*0.77*	*2.69 (2.20, 3.19)*	*60.7 (47.4, 77.7)*	*136 (99.8, 186)*	*61.9 (58.7, 65.2)*	*83.8 (58.9, 119)*
Q2 [1.77-2.90]	*2.45*	*2.89 (2.45, 3.31)*	*53.7 (43.3, 66.6)*	*138 (105, 179)*	*65.4 (63.3, 67.5)*	*88.6 (67.0, 117)*
Q3 [3.00-4.30]	*3.40*	*2.59 (2.13, 3.05)*	*36.7 (26.8, 50.3)**	*81.5 (57.7, 115)**	*59.3 (55.6, 62.9)*	*47.4 (31.8, 70.6)**
Q4 [4.40-12.1]	*5.80*	*3.11 (2.47, 3.75)*	*31.2 (22.7, 42.8)**	*81.8 (56.1, 119)**	*59.4 (66.7, 63.0)*	*47.6 (31.3, 72.6)**
p, trend		*0.48*	*0.0005*	*0.01*	*0.11*	*0.01*

*= $p \leq 0.05$

(a): Data are presented as predicted estimates (95% CI) adjusted for BMI, smoking status, alcohol drinker, season, and abstinence time at the mean level of continuous covariates and adjusted for frequency of categorical measures. Motile sperm and total motile sperm count models were further adjusted by time to start semen analysis.

(b): Further adjustment for NDL-PCBs concentrations.

Minguez-Alarcon et al 2017 – main conclusions

- Association with TCDD, PCDD-TEQs, PCDD/F-TEQs
- No association with PCDF-TEQs, DL-PCB-TEQs, Total TEQs
- Cannot distinguish pre-and postnatal exposure
- Sensitive period is not known

Congener pattern will be discussed in a later presentation

Outline

- Outcomes in humans – other than semen quality
- Semen quality
  - Seveso cohorts
  - Russian Children's Study (RCS)
  - Factors for consideration:
    - ✓ Organochlorine pesticides
    - ✓ Pubertal development
    - ✓ Loss to follow-up
    - ✓ Lead
    - ✓ Alcohol and tobacco consumption
    - ✓ Variability in semen analyses
  - Dose-response assessment
  - Causality of decreased sperm concentration
- Relevance of impaired semen quality

OCP levels in Russian Children's Study – confounding?

Predictors of Serum Chlorin… Concentrations among Prepubertal Russian Boys

Thuy Lam,[1] Paige L. Williams,[2] … ick,[1,5] Mary M. Lee,[6] Linda S. Birnbaum,[7,8] Boris R… Jr.,[12,13,14] Wayman E. Turner,[15] and Russ Hauser[1]

HCB: "25-percentile being approximately 8-fold higher than the median level in the US"

Table 2. Distribution of measured OCP… …ned in the Russian Children's Study ($n = 355$).[a]

		Percentile						
OCP	*n*	Minimum	10th	25th	50th (median)	75th	90th	Maximum
HCB	355	32	80	107	158	246	364	2,660
β-HCH	355	39	81	112	167	270	412	2,860
p,p′-DDE	355	49	122	187	284	492	835	9,370

[a]No values < LOD.

Table 3. Median OCP concentrations (ng/g lipid) in 8- to 9-year-old boys in the Russian Children's Study compared with other pediatric studies.

Country	Year	*n*	Age range (years)	Population	HCB	β-HCH	*p,p′*-DDE
Russia (current study)	2003–2005	355	8–9	Boys	158	167	284
USA (NHANES)[a]	2003–2004	588	12–19	Boys and girls	13.4	< LOD	93.6
Belgium[b]	2003–2004	1,679	14–15	Boys	22.8	—	104
Faroe Islands[c]	1986–1987	788	14	Boys and girls	—	—	467
Slovakia[d] (contaminated Michalovce district)	2001	216	8–10	Boys and girls	79.6	—	344

Abbreviations: —, OCP not measured; NHANES, National Health and Nutrition Examination Survey. LOD = 7.8 ng/g lipid.
[a]Patterson et al. (2009). [b]Den Hond et al. (2011). [c]Barr et al. (2006). [d]Petrik et al. (2006).

Correlation with TEQ
High (β-HCH)
Moderate (DDE, HCB)

Tables: T. Lam et al 2013. Environ Health Perspect 121:1372–1377; http://dx.doi.org/10.1289/ehp.1306480

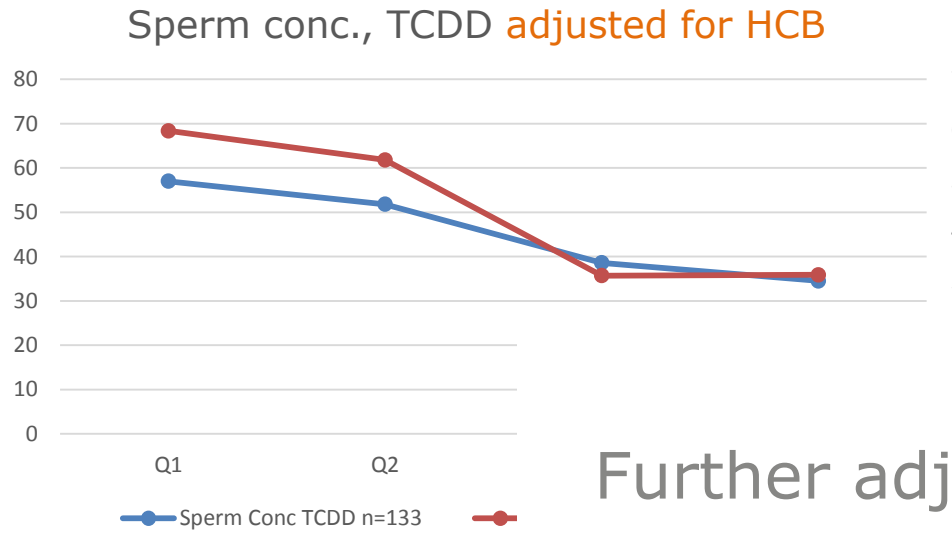
OCPs not associated with sperm concentration

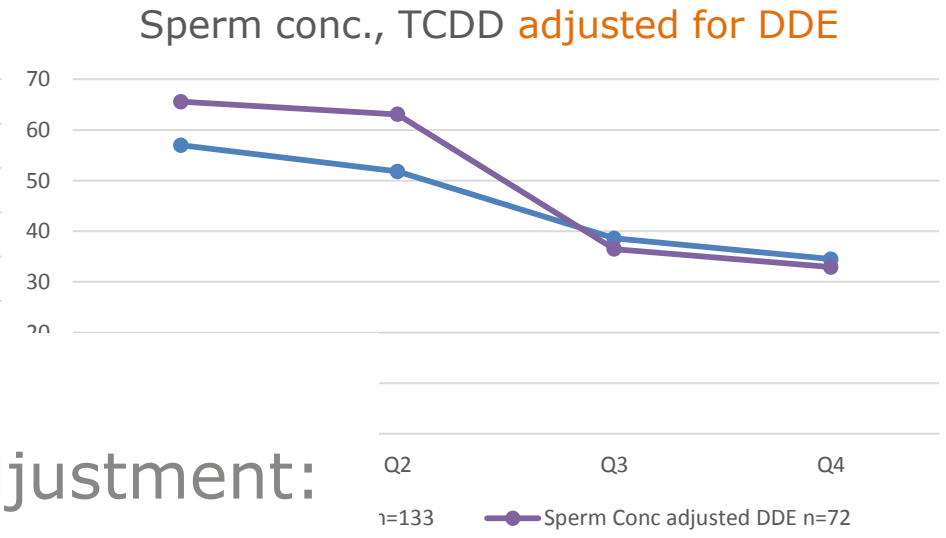
	Sperm Concentration (mill/mL)
HCB (pg/g serum)	
Low [283-729]	50.9 (42.2, 61.4)
High [732-15482]	52.9 (41.4, 67.6)
p, value	0.81
βHCH (pg/g serum)	
Low [222-803]	51.0 (42.1, 61.7)
High [812-13732]	52.8 (41.4, 67.3)
p, value	0.82
p,p´-DDE (pg/g serum)	
Low [370-1360]	48.9 (39.2, 61.1)
High [1388-27437]	55.1 (44.4, 68.2)
p, value	0.45

Chlorinated pesticides (HCB, βHCH, DDE)
not associated with sperm concentration

L. Mínguez-Alarcón, J Burns, R. Hauser, 2017-2018, documentation submitted to EFSA

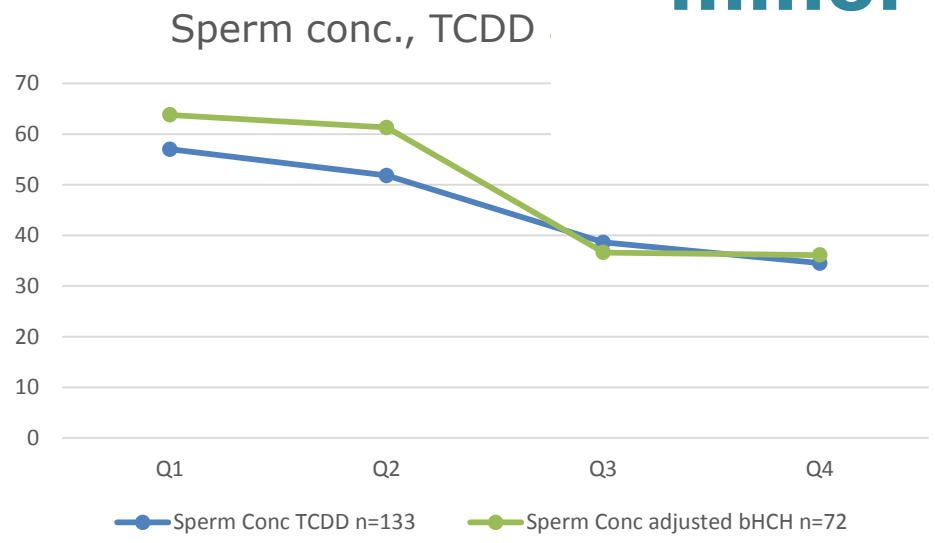
Sperm concentration, adjusted for NDL-PCBs and OCPs

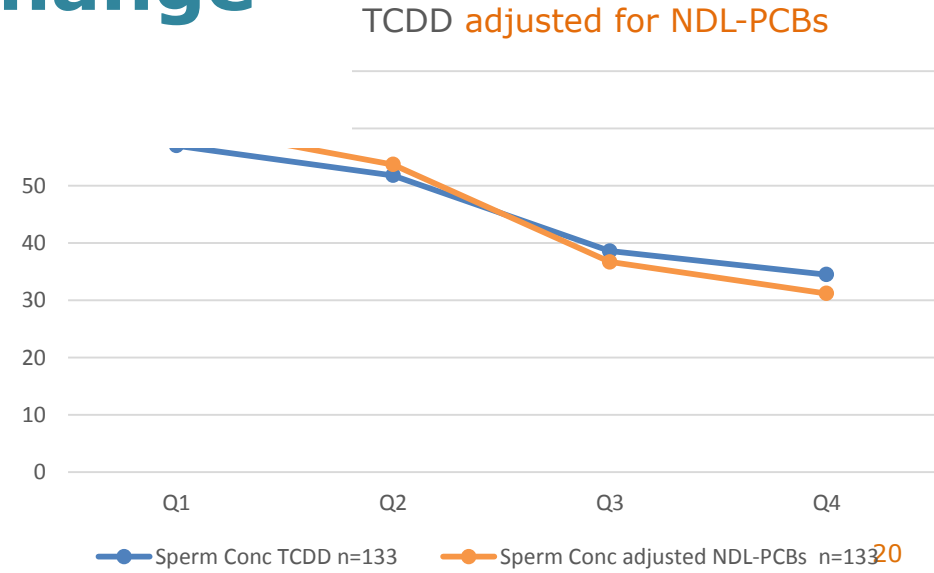




Further adjustment:

minor change





Can delayed puberty affect sperm counts at age 18-19 y?

- Russian Children's Study: Sexual maturation assessed at age 17-18 and sperm collected at age 18-19
- Studies on puberty development in relation to PCDD/Fs+DL-PCBs, NDL-PCBs, OCPs
- Puberty timing not used as critical endpoint
  - Although similar effect in rodents
  - One cohort
  - Possible confounding by OCPs

Effect of puberty timing on semen quality is unknown

PCDD/Fs and PCBs, pubertal development, RCS

- Tanner staging (visual inspection)
  - Genitalia: Onset G2, Maturity G5
  - Pubarche: Onset P2, Maturity P5
- Testicular volume (objective measure)
  - Onset: TV>3ml, Maturity: TV>20ml
- Yearly examination from age 8-9 to age 17-18
  - 67% retention rate
  - Boys who stayed in study had lower BMI at inclusion and parents with longer education
- Three papers:
  - Korrick 2011: Levels in boys and pubertal **onset**, assesed at age 11-12
  - Humblet 2011: Maternal levels and pubertal **onset**, assesed at age 11-12 (no association)
  - **Burns 2016: Levels in boys and pubertal timing (from onset to sexual maturity) from age 8-9 to age 17-18**

Delay in puberty with PCDD/Fs+DL-PCBs, Burns 2016

Associations of Peripubertal Serum Dioxin and Polychlorinated Biphenyl Concentrations with Pubertal Timing among Russian Boys

Jane S. Burns,[1] Mary M. Lee,[2,3] Paige L. Williams,[4,5] Susan A. Korrick,[1,6] Oleg Sergeyev,[7,8] Thuy Lam,[1,9] Boris Revich,[10] and Russ Hauser[1,5]

- At 17-18 years: 100% had entered puberty, 95-97% finished puberty.
- PCDD/Fs and DL-PCBs (TEQ) and non-DL-PCBs in blood at age 11-12 have divergent associations with puberty: models were adjusted for both
- Dose-related association between total WHO_{2005}-TEQ, adjusted for NDL-PCBs
  - **delayed pubertal onset**. Q4 vs Q1: 11.6 months
  - **delayed sexual maturity**. Q4 vs Q1: 11.6 months
- Stayed significant when not adjusted for NDL-PCBs
- NDL-PCBs in the boys' blood at age 8-9 years was associated (but not significantly) with **earlier pubertal onset**, after adjustment for TEQ

Delay in puberty, mean shift in months, Burns 2016

Pubertal onset, adjusted for NDL-PCBs

Serum quartile	Testicular volume > 3 mL[a] Mean shift (95% CI)	*p*-Value
ΣTEQs, adjusted for Σnon-dioxin-like PCBs[d]		
Q1	Reference	
Q2	4.0 (–1.9, 9.8)	0.19
Q3	7.5 (0.6, 14.4)	0.03
Q4	11.6 (3.8, 19.4)	0.004
Trend test[e]		0.003

Not adjusted for NDL-PCBs

Serum Quartile	Testicular Volume > 3 mL[a] Mean shift (95% CI)	P-value
ΣTEQs[d]		
Q1	Reference	
Q2	1.9 (-3.4, 7.3)	0.48
Q3	3.2 (-2.2, 8.6)	0.24
Q4	5.6 (0.3, 10.9)	0.04
Trend test[g]		0.04

Sexual maturity adjusted for NDL-PCBs

Serum quartile	Testicular volume ≥ 20 mL[a] Mean shift (95% CI)	*p*-Value
ΣTEQs, adjusted for Σnon-dioxin-like PCBs[d]		
Q1	Reference	
Q2	6.0 (1.6, 10.5)	0.008
Q3	8.8 (3.7, 14.0)	< 0.001
Q4	11.6 (5.7, 17.6)	< 0.001
Trend test[e]		< 0.001

Significantly different from Q2

Not adjusted for NDL-PCBs

Serum Quartile	Testicular Volume ≥ 20 mL[a] Mean shift (95% CI)	P-value
ΣTEQs[d]		
Q1	Reference	
Q2	4.2 (0.1, 8.2)	0.04
Q3	6.1 (2.0, 10.2)	0.003
Q4	7.7 (3.6, 11.8)	<0.001
Trend test[g]		<0.001

(Unadjusted from online supplemental)

RCS: Correlation with organochlorines

Spearman correlation coefficients between organochlorine compounds and blood lead						
	∑TEQs	∑NDL-PCBs	∑DLCs	β-HCH	p,p'-DDE	HCB
∑TEQs	1.00 (n=468)	0.82 (n=468)	0.80 (n=468)	0.72 (n=350)	0.51 (n=350)	0.53 (n=350)
∑NDL-PCBs	0.82 (n=468)	1.00 (n=468)	0.74 (n=473)	0.71 (n=350)	0.61 (n=350)	0.49 (n=350)
∑DLCs	0.80 (n=468)	0.74 (n=468)	1.00 (n=468)	0.58 (n=350)	0.42 (n=350)	0.48 (n=350)
β-HCH	0.72 (n=350)	0.71 (n=350)	0.58 (n=350)	1.00 (n=350)	0.61 (n=350)	0.54 (n=350)
p,p'-DDE	0.51 (n=350)	0.61 (n=350)	0.42 (n=350)	0.61 (n=350)	1.00 (n=350)	0.34 (n=350)
HCB	0.53 (n=350)	0.49 (n=350)	0.48 (n=350)	0.54 (n=350)	0.34 (n=350)	1.00 (n=350)
Based on analysis datasets						

High
Moderate

L. Mínguez-Alarcón, J Burns, R. Hauser, 2017-2018, documentation submitted to EFSA

OHCs and delayed puberty (Lam et al. 2015)

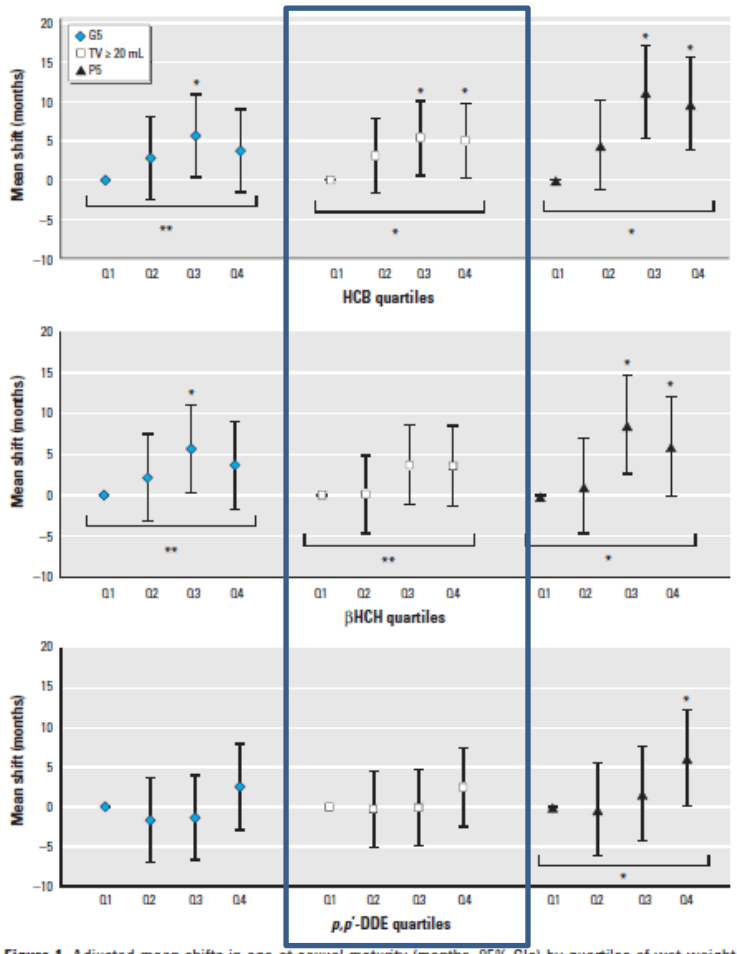


Figure 1. Adjusted mean shifts in age at sexual maturity (months, 95% CIs) by quartiles of wet-weight serum OCP concentrations among 350 Russian boys, relative to the lowest quartile (Q1). Baseline covariates for each model are as follows: G5: boy's total serum lipids, macronutrients (total caloric intake, percent calories from dietary carbohydrates, fat, protein) (missing macronutrients, $n = 3$); TV ≥ 20 mL: boys' total serum lipids, birth weight, blood lead levels, biological father's absence from the household (missing birth weight, $n = 1$); P5: boys' total serum lipids, biological father's absence from the household. HCB wet-weight quartiles (pg/g serum): Q1, 169–516; Q2, 517–751; Q3, 752–1,156; Q4, 1,157–15,482. βHCH wet-weight quartiles (pg/g serum): Q1, 209–567; Q2, 568–814; Q3, 815–1,294; Q4, 1,295–13,732. *p,p'*-DDE wet-weight quartiles (pg/g serum): Q1, 261–907; Q2, 908–1,406; Q3, 1,407–2,327; Q4, 2,328–41,301.
*$p \leq 0.05$. **$p \leq 0.10$.

Environ Health Perspect. 2015 Nov;123(11):1216-21. doi: 10.1289/ehp.1409022. Epub 2015 May 22.

Prepubertal Serum Concentrations of Organochlorine Pesticides and Age at Sexual Maturity in Russian Boys.

Lam T[1], Williams PL, Lee MM, Korrick SA, Birnbaum LS, Burns JS, Sergeyev O, Revich B, Altshul LM, Patterson DG Jr, Hauser R.

https://ehp.niehs.nih.gov/doi/pdf/10.1289/ehp.1409022

Caused by PCDD/Fs and DL-PCBs or OCPs?

No adjustment for TEQ in the papers

Environ Int. 2014 Dec;73:135-42. doi: 10.1016/j.envint.2014.06.020. Epub 2014 Aug 10.

Prepubertal organochlorine pesticide concentrations and age of pubertal onset among Russian boys.

Lam T[1], Williams PL[2], Lee MM[3], Korrick SA[4], Birnbaum LS[5], Burns JS[6], Sergeyev O[7], Revich B[8], Altshul LM[9], Patterson DG Jr[10], Turner WE[11], Hauser R[12].

https://www.ncbi.nlm.nih.gov/pubmed/25118086

Delayed puberty - consequence for the TWI?

- If pubertal timing is associated with sperm concentration, would it have had consequences for the TWI?
  - Probably not
  - **Delay in puberty is an adverse effect**
  - Significant delay from second quartile (TEQ)
  - Quartiles quite similar in Burns 2016 and Mínguez-Alarcón 2017

	N	Q1 TEQ	Q2 TEQ	Q3 TEQ	Q4 TEQ
Mínguez-Alarcón 2017 (semen quality)	133	4.9-17	17-21	22-33	33-107
Burns 2016 (pubertal development)	473	4.0-15	15-21	21-33	33-175

- Reduced growth was associated with exposure to both PCDD/Fs and NDL-PCBs → confounder of delayed pubertal onset?

Conclusions - OCPs and puberty timing and sperm conc

- Reduction of sperm concentrations are not associated with OCPs
- Delay in puberty development (adverse effect) may be caused by exposure to PCDD/Fs and DL-PCBs (TEQs)
- Effect of puberty timing on semen quality is unknown

Loss to follow-up at age 17-18 (Mínguez-Alarcón 2017)

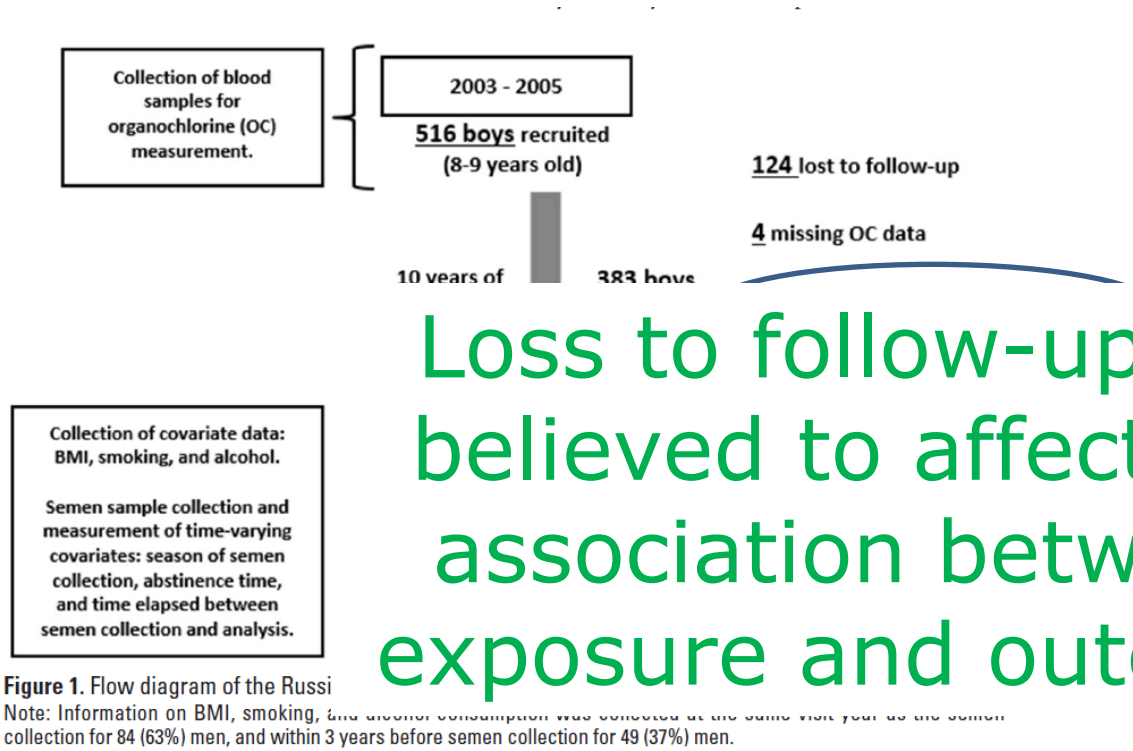


Figure 1. Flow diagram of the Russi
Note: Information on BMI, smoking, and alcohol consumption was collected at the same visit year as the semen collection for 84 (63%) men, and within 3 years before semen collection for 49 (37%) men.

From Burns et al. 2016:

"Height z-scores, and most demographic characteristics did not differ significantly between boys who remained in the study and
o dropped out
e visit at 17–18

o remained in the
re leaner at
(mean BMI z-
.34 vs. –0.05),
and more likely to have postsecondary school–educated parents (95% vs. 87%)."

Loss to follow-up not believed to affect the association between exposure and outcome

Quite similar exposure (serum levels):

Group with puberty data (Burns 2016):
482 boys **median 21.1 pg** TEQ/g fat (IQR 14.4-33.2)

Group with sperm data (Mínguez-Alarcón 2017):
133 boys **median 21.9 pg** TEQ/g fat (IQR 16.8-33.3)

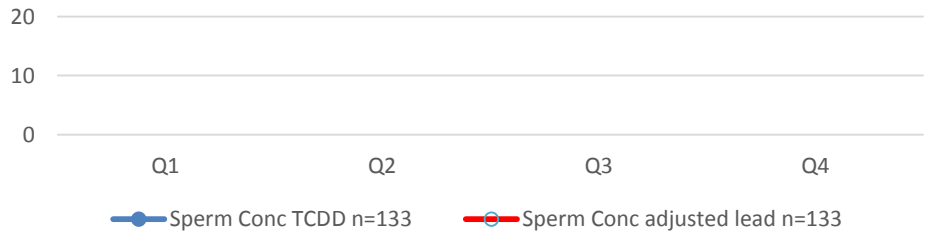
Lead in the Russian Children's Study

- The median blood lead concentration in the boys at age 8–9 was 3 µg/dL (Hauser et al. 2008), which is not much higher than NHANES
- Lead in blood was not correlated with TCDD or PCDD-TEQs
- Reason to believe lead is equally distributed across quartiles of TCDD
- Adjustment for lead in blood did not change the associations between TCDD and sperm parameters

RCS: Correlation with lead at age 11-12, and adjustment

Spearman correlation coefficients between organochlorine compounds and blood lead						
	∑TEQs	∑NDL-PCBs	∑DLCs	β-HCH	p,p'-DDE	HCB
Blood lead	0.18 (n=468)	0.19 (n=468)	0.15 (n=473)	0.16 (n=350)	0.24 (n=350)	0.10 (350)

Blood lead levels are not believed to affect the association between exposure and outcome



L. Mínguez-Alarcón, J Burns, R. Hauser, 2017-2018, documentation submitted to EFSA

Alcohol and tobacco consumption, RCS

- Information about smoking and alcohol is not collected in an optimal way
- Although some studies shows significant associations these variables are not strongly associated neither with semen quality nor with dioxins (Reviews by Dai et al. (2015) Asian J Andrology and Ricci et al. (2016) RBM online)
- To be a confounder the variable should be associated with both the exposure and the outcome

Although not optimal information on these variables, it will most probably not change the conclusion

Within- and between variability in sperm concentrations

Relevance for the Russian Children's Study

- It is true that there is within-person variability in sperm quality, for example due to abstinence time
- Typically the CV for sperm concentration within individuals at repeated samples is 40–50% (Poland 1985, Keel 2005)
- In the **RCS two samples were collected** from each participant and the **CV for these was 48%, so "normal"**
- **Between-individual variability is considerably higher** with typical intraclass correlation (ICC; s2-between/s2-total) 75-90% (Poland 1985, Keel 2005, Chiu 2017)
- Due to the expected high ICC, the number of participants (N=133) is **not considered too low** to detect the impact of an external factor
- **Misclassification** due to within-person variability is expected to be **non-differential**, so a true association should be attenuated (a minus sign in the Uncertainty section)

Outline

- Outcomes in humans – other than semen quality
- Semen quality
  - Seveso cohorts
  - Russian Children's Study (RCS)
  - Factors for consideration:
    - ✓ Organochlorine pesticides
    - ✓ Pubertal development
    - ✓ Loss to follow-up
    - ✓ Lead
    - ✓ Alcohol and tobacco consumption
    - ✓ Variability in semen analyses
  - Dose-response assessment
  - Causality of decreased sperm concentration
- Relevance of impaired semen quality

Dose-response assessment, deciles

- We looked into the possibility of dividing the data into deciles.
- The number of individuals is too low to use deciles in the dose-response analyses.
- It was decided to use the published data in quartiles.
- Possible that the lowest quartile in Russian Children's Study is not a true NOAEL.

Reduced sperm concentration – CAUSALITY

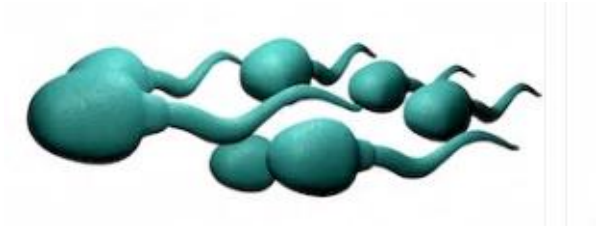
- Seen in **3 cohorts** (2 Seveso cohorts + Russian Children's Study)
- Dose-response pattern looks similar across cohorts (steep decline and leveling off at 40-50% reduction)
  - Uncertainty in background exposure, timing of exposure and congener composition in Seveso makes direct comparison of dose-response between Seveso and RCS difficult
- Sensitive endpoint also in **rodents** (basis SCF TWI)
- Seen also in mice expressing constitutive active AhR

→ Ah-R mediated, although MoA not known

- Similar dose-response pattern in rodents

Biological relevance - DECREASED SEMEN QUALITY

- Humans have lower sperm production than other species, making **humans more sensitive**
  - Rat: 17 mill sperm/g testis/day
  - Human: 4 mill sperm/g testis/day



- Reduction in semen quality can lead to **increased time to pregnancy and infertility, clear adverse effects**
- Decrease in population mean will increase % infertile at population level
  - Below 40 mill/mL associated with increased time to pregnancy (Bonde et al. Lancet, 1998)
  - Below 55 mill/mL associated with increased time to pregnancy (Slama et al. Human Reproduction 17, 2002)
- Semen quality affected by several factors

Exposure to PCDD/Fs and DL-PCBs probably one of the factors contributing to decreasing sperm counts