International Symposium （Kushiro, Nov. 13. 2006)

Exposure to Endocrine Disrupting Chemicals and its Adverse Effects on the Next Generation: Research Approaches and Future Issues

–based on the results of "the Hokkaido Study of Environment and Children's Health,

Reiko Kishi, MD & PhD, MPH
Department of Public Health Sciences
Graduate School of Medicine
Hokkaido University

points
for risk assessments

(1) What is the most sensitive health effects in the medical sense?

(2) Distinguish hazards and risks

- **What are risks?**
  - **①certain populations**
    (for example, Japanese pregnant women and children)
  - **②the exposure level**
  - **③getting health problems ?**

(3) How reliable is the evidence?

- **Based on epidemiological causal relationship**
  - time relations、magnitudes of risks、specificity、biological plausibility
  - accurate exposure measurements
    (level of chemicals and measuring method)
  - validity of outcomes

(4) possible only with substantial epidemiological data

Study Questions on the Effects of Endocrine Disruptors on the Next generation

((PCBs, Dioxins, Pesticides, Phtalates)

1. Urogenital Anomalies ?
2. Thyroid Hormone ?
3. Poorer Motor/Cognitive Development ?
4. Infantile Allergies ?

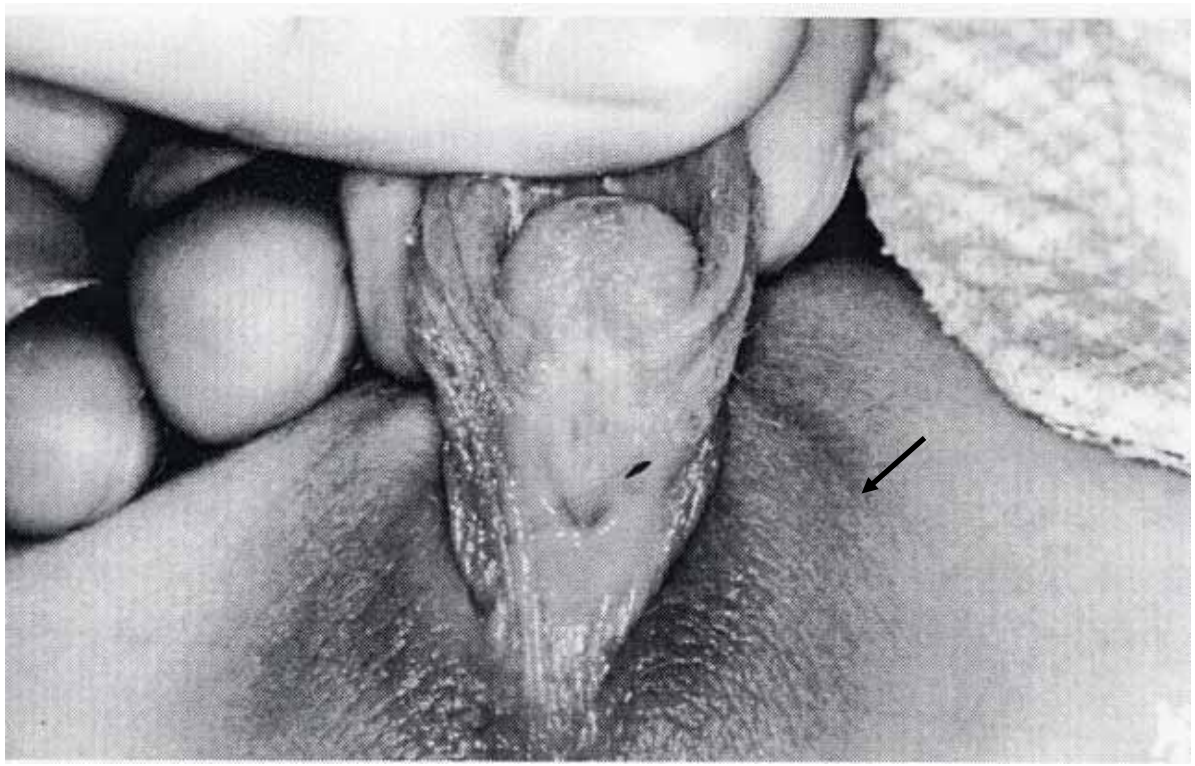
①. **Is the number of congenital anomalies really increasing?** (If so, are exogenous endocrine disrupting chemicals causing this?)

② **Is there influence on neurobehavioural development of children of general public with background level?**

3. **Do PCBs and dioxins influence thyroid function of children and pregnant women?**

4. **Is there an association between Environmental chemicals and allergic disorders** which is increasing in number in recent years?

Hypospadias
(尿道下裂)

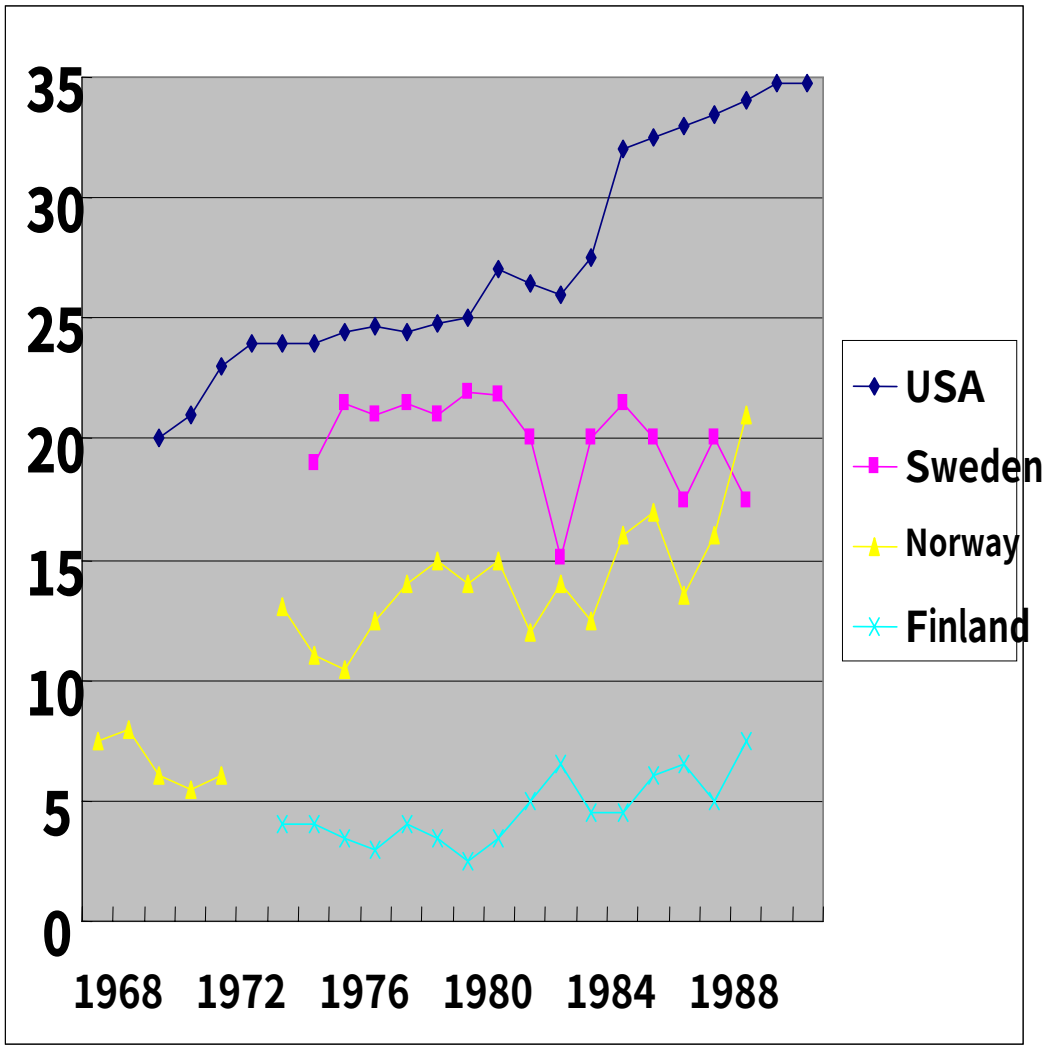


A common congenital anomaly

The urethral opening is ventral surface of the penis, or on the scrotum, or perineum

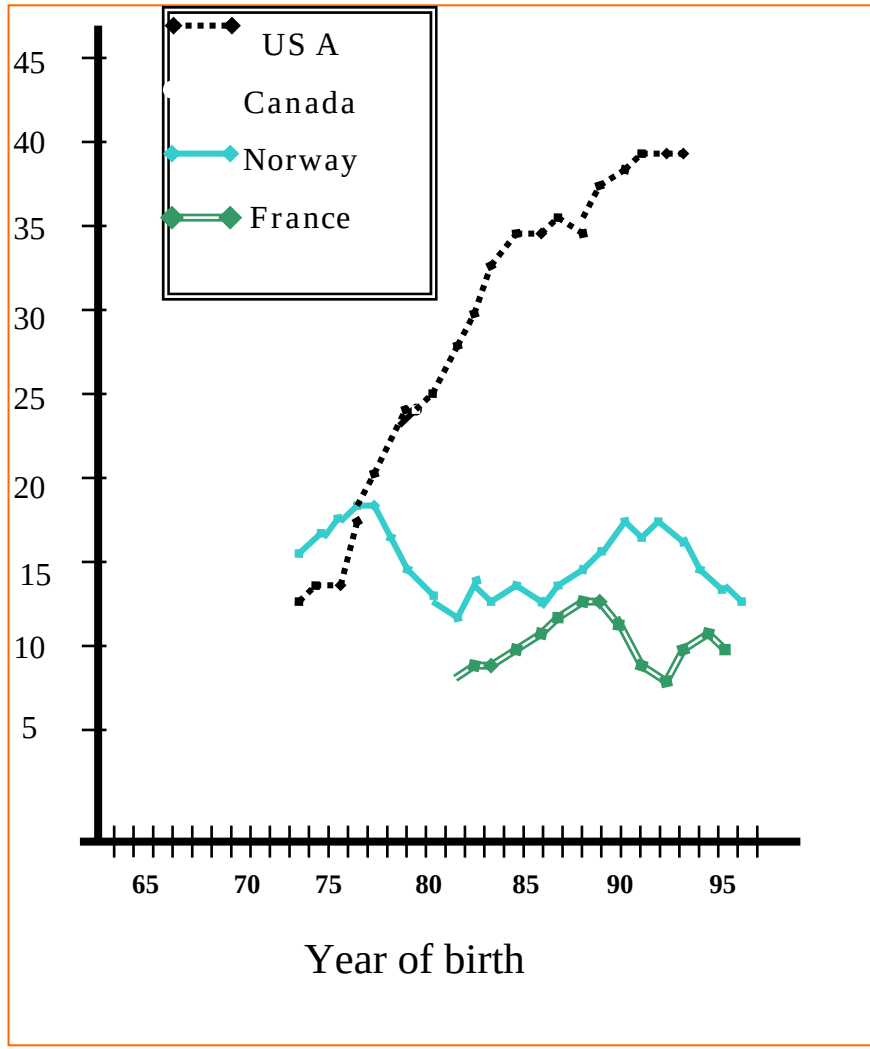
Department of Renal and Genitourinary Surgery,
Hokkaido University Hospital

Transition of Incidence of Hypospadias(尿道下裂)



Toppari et al.（2001)

Transition of Incidence of Cryptorchidism (停留精巣)



Paulozzi (1999)

Environmental Factors of Hypospadias ?
in Previous Epidemiological Studies

Case-Control Study

agricultural chemicals	No association

Cohort Studies

PCB	No data available
DDE	No association with the concentration of DDE in maternal serum during pregnancy.
DES	The prevalence ratio is increased with gestational DES exposure
Phytoestrogens	Increasing odds ratio in vegetarian during pregnancy.

Risk Factors for Hypospadias
尿道下裂 (Sapporo)

Characteristics	Cases (%)	Control (%)	Adjusted OR	95%CI
Weight at birth <2500g	22.8	6.1	4.8	1.8-12.7
gestational age <36	38.7	14.2	4.8	2.0-10.8
SGA	54.5	23.0	4.9	2.4-10.2
cesarean section	40.7	17.0	3.3	1.7-6.3
pregnancy toxicosis	27.1	9.2	7.1	1.8-28.1

Adjusted for maternal age ,infant birth year, and household income

Risk Factors for Hypospadias 尿道下裂 (Sapporo)

Characteristics	Cases (%)	Control (%)	Adjusted OR	95%CI
Antiepileptic drug common user	0.9	0	∞	
Paternal smoking during early pregnancy	71.9	64.0	1.4	0.8-2.4
Paternal smoking history	71.9	64.0	1.4	0.8-2.4
Maternal smoking during early pregnancy	30.7	28.1	1.1	0.6-2.0
Maternal smoking history	30.7	28.1	0.9	0.5-1.5

Adjusted for maternal age ,infant birth year, and household income

Risk Factors for Cryptorchidism 停留精巣 (Sapporo)

Characteristics	Cases (%)	Control (%)	Adjusted OR	95%CI
Abnormal labor (Cesarean section、Vacuum extraction/ forceps delivery)	26.4	13.9	2.3	1.2-4.4
Paternal smoking during early pregnancy	75.3	59.6	1.9	0.98-3.5
Paternal smoking during pregnancy	73.6	59.6	2.0	1.1-3.7
Paternal exposures to diesel exhaust during early pregnancy	17.0	8.0	2.5	1.0-6.2
Maternal smoking during early pregnancy	33.0	29.6	1.0	0.6-2.0
Maternal smoking during pregnancy	19.3	19.1	1.0	0.5-2.0

Adjusted for maternal age ,infant birth year, and household income

Several Limitations of the Case Control Study as a "Retrospective Study"

- **"Selection bias"** in hospitals in many countries with no registry system for birth defects.
- Maternal **"Recall bias"** since mothers have to recall their past pregnancy for questionnaire
- **"Selection bias"** due to the difficulty of obtaining compliance of controls
- **Temporal causal relationship is unclear**
- **Difficult to accurately assess exposure levels** because of the difficulty of obtaining **biological samples** (such as blood)


Sapporo
Kushiro
北海道
“The Hokkaido Study of Environment and Child Health”
Tokyo
Sapporo
Kushiro

－Study Design －

① The focus is placed on the effects in the general public with background level

② Prospective cohort study to obtain data on various environmental factors during the fetal stages.

③ Stored biological samples for a certain period, and after following offspring, risks are assessed for congenital anomalies, disorders in neurobehavioral development, deterioration in thyroid functions, and immune and allergic disorders.

④ Accurate exposure measurements.

⑤ Study on predisposing factors of individual susceptibility from the viewpoint of preventive medicine to identify high-risk groups.

Prevalence of congenital anomaly

(from the Hokkaido Study of Environment and Children's Health)

congenital anomaly	# of Samples	*Rate of occurences in Hokkaido (per 10000 born)	** Rate of JAOG occurrences
Congenital heart disease	28	39.1	–
Ventricular septal defect	14	19.5	22.3
Atrial septal defect	6	8.4	7.2
Tetralogy of Fallot	4	5.6	5.3
Down syndrome	11	15.4	12.0
Cleft lip and palate	9	12.6	13.6
Hydronephrosis	8	11.2	–
Polydactyly	7	9.8	7.0
Accessory ear	6	8.4	–
Crytorchidism	6	16.8	(70)[1]
Anencephalia	4	5.6	–
Cleft palate	4	5.6	3.3
Diaphragmatic hernia	4	5.6	5.3
Fetal edema	4	5.6	–
(reference) Hypospadias	2	5.6	4.3

*2003.1-2005.5 7164人, **1997-2001 only within top 20th, [1]Occurence rate in Korea 0.7%

Effects of Maternal Folic Acid in Serum on Infant Birth Weight*

Folic Acid in Serum (ng/ml)	standard partial regression coefficient (β)	*P* Value
2.4～5.6	-0.042	0.02
5.7～6.8	-0.015	0.42
6.9～8.3	standard	—
8.4～37.0	-0.007	0.72

***multiple regression analysis**
Adjusted for gestational age, infant gender, birth history, maternal BMI before pregnancy, maternal age at delivery, maternal education, and maternal smoking during pregnancy

Maternal smoking and Folic Acid Level in Serum

Smoking status	Number of samples (%)	Folic Acid in Serum (Mean±SD)	*P* Value *
Non-smoking	**2099 (63.7%)**	**7.5±2.6**	**–**
quitting smoking	**697 (21.2%)**	**6.9±2.3**	**＜0.01**
smoking	**499 (15.1%)**	**6.6±2.1**	**＜0.01**

*Mann-Whitney test with Bonferroni's correction was used to compare with non-smoking group

Characteristics influencing on Maternal Folic Acid Level in Serum *

Characteristics	standard partial regression coefficient （β）	*P* Value
Maternal age at deliverly	0.097	＜0.01
Maternal smoking during pregnancy	-0.112	＜0.01
Maternal BMI before pregnancy	-0.025	0.16
Maternal education	0.024	0.19

*multiple regression analysis

Results at this point, prospective Cohort Study ①

- Influences of folic acid levels and smoking during early pregnancy on birth weight and developmental retardation inside uterus (IUGR) are found.

On going ,,,,,,

① various environmental factors associate with congenital anomalies ?
②Interactive effect of environmental factors and predisposing factors of individual genetic susceptibility of Japanese mothers and children ?

Cohort study ②

Study child neurobehavioral development in detail after birth by inviting participation from mothers in middle to late pregnancy (23 - 35 weeks).

(children born in one of maternity hospital in Sapporo city)

Exposure Measurements

- **The Concentration of PCBs and dioxins in the maternal blood were measured using high-resolution gas chromatography/ high-resolution mass spectrometry (HRGC/HRMS) at Fukuoka Institute of Health and Environmental Sciences.**

- **The levels of PCBs and dioxins were measured in each isomaer [7 polychlorinated dibenzo-*p*-dioxins (PCDDs), 10 polychlorinated dibenzofurans (PCDFs), 4 non-*ortho* PCBs, 8 mono-*ortho* PCBs, 2 di-*ortho* PCBs], and the total toxicity equivalency quality (TEQ) levels were calculated.**

- **For 64 subjects, 68 PCBs were also measured to compare with data from other countries.**

MDI and PDI Scores for Infants in Relation to the Level of Isomers of PCBs and Dioxins in Maternal Blood①

	MDI				PDI			
	β	*t*	*p*		*β*	*t*	*p*	
＜PCDD＞								
2,3,7,8-TCDD	-0.150	-1.714	0.089		-0.105	-1.235	0.219	
1,2,3,7,8-PeCDD	0.067	0.771	0.442		-0.036	-0.423	0.673	
1,2,3,4,7,8-HxCDD	-0.035	-0.394	0.694		-0.124	-1.462	0.146	
1,2,3,6,7,8-HxCDD	0.023	0.259	0.796		-0.045	-0.520	0.604	
1,2,3,7,8,9-HxCDD	0.002	0.026	0.979		-0.189	-2.284	0.024	*
1,2,3,4,6,7,8-HpCDD	-0.219	-2.395	0.018	*	-0.240	-2.749	0.007	**
OCDD	-0.173	-1.864	0.065		-0.172	-1.927	0.056	
＜PCDF＞								
2,3,7,8-TCDF	-0.050	-0.584	0.560		-0.178	-2.175	0.031	*
1,2,3,7,8-PeCDF	0.014	0.158	0.875		-0.196	-2.412	0.017	*
2,3,4,7,8-PeCDF	0.022	0.252	0.801		-0.046	-0.544	0.588	
1,2,3,4,7,8-HxCDF	-0.107	-1.199	0.233		-0.137	-1.615	0.109	
1,2,3,6,7,8-HxCDF	-0.099	-1.117	0.266		-0.167	-1.990	0.049	*
2,3,4,6,7,8-HxCDF	0.026	0.302	0.763		-0.167	-2.012	0.046	*
1,2,3,7,8,9-HxCDF	ND	ND	ND		ND	ND	ND	
1,2,3,4,6,7,8-HpCDF	-0.042	-0.482	0.631		-0.064	-0.763	0.447	
1,2,3,4,7,8,9-HpCDF	ND	ND	ND		ND	ND	ND	
OCDF	-0.057	-0.656	0.513		-0.032	-0.390	0.697	

ND：non-detectable，Multiple-regression analysis.

The level of PCBs and dioxins in maternal blood were logarithmically transformed.Adjusted for gestational age, smoking suring pregnancy, and blood sampling time. * $p < 0.05$; ** $p < 0.01$

MDI and PDI Scores for Infants in Relation to the Level of Isomers of PCBs and Dioxins in Maternal Blood②

	MDI			PDI		
	β	*t*	*p*	*β*	*t*	*p*
＜non-ortho PCB＞						
33'4'4'-TCB(#77)	0.035	0.405	0.686	-0.007	-0.082	0.935
344'5-TCB(#81)	ND	ND	ND	ND	ND	ND
33'44'5-PenCB(#126)	-0.005	-0.056	0.956	-0.106	-1.277	0.204
33'44'55'-HxCB(#169)	0.008	0.091	0.928	-0.075	-0.898	0.371
＜mono-ortho PCB＞						
233'44'-PenCB(#105)	-0.007	-0.082	0.935	-0.090	-1.083	0.281
2344'5-PenCB(#114)	-0.030	-0.348	0.729	-0.110	-1.325	0.187
23'44'5-PenCB(#118)	-0.020	-0.232	0.817	-0.111	-1.334	0.185
2'344'5-PenCB(#123)	0.032	0.367	0.714	-0.081	-0.970	0.334
233'44'5-HexCB(#156)	-0.007	-0.077	0.939	-0.077	-0.932	0.353
233'44'5'-HexCB(#157)	-0.045	-0.519	0.604	-0.126	-1.521	0.131
23'44'55'-HexCB(#167)	-0.018	-0.211	0.833	-0.112	-1.353	0.178
233'44'55'-HpCB(#189)	-0.106	-1.232	0.220	-0.117	-1.420	0.158
＜di-ortho PCB＞						
22'33'44'5-HpCB(#170)	-0.033	-0.386	0.700	-0.110	-1.327	0.187
22'344'55'-HpCB(#180)	-0.030	-0.349	0.728	-0.074	-0.891	0.374

ND：non-detectable，Multiple-regression analysis.

The level of PCBs and dioxins in maternal blood were logarithmically transformed. Adjusted for gestational age, smoking suring pregnancy, and blood sampling time. * $p < 0.05$; ** $p < 0.01$

MDI and PDI Scores for Infants in Relation to the Level of PCBs and Dioxins (Total and TEQ values) in Maternal Blood

	MDI			PDI		
	β	*t*	*p*	*β*	*t*	*p*
＜Total＞						
Total PCDD	-0.166	-1.788	0.076	-0.173	-1.935	0.055
Total PCDF	-0.041	-0.467	0.641	-0.117	-1.396	0.165
Total PCDD/PCDF	-0.163	-1.756	0.081	-0.173	-1.935	0.055
Total Non-ortho PCBs	0.007	0.076	0.939	-0.077	-0.921	0.359
Total Mono-ortho PCBs	-0.019	-0.224	0.823	-0.111	-1.334	0.184
Total Coplanar PCB	-0.014	-0.166	0.869	-0.090	-1.091	0.277
Total	-0.016	-0.179	0.858	-0.092	-1.111	0.269
＜WHO-98＞						
Total PCDDs-TEQ	0.008	0.088	0.930	-0.068	-0.796	0.427
Total PCDFs-TEQ	0.002	0.020	0.984	-0.071	-0.837	0.404
Total PCDDs/PCDFs-TEQ	0.007	0.074	0.941	-0.069	-0.810	0.420
Total Non-ortho PCBs-TEQ	-0.002	-0.023	0.982	-0.104	-1.248	0.214
Total Mono-ortho PCBs-TEQ	-0.017	-0.196	0.845	-0.103	-1.244	0.216
Total Coplanar PCBs-TEQ	-0.006	-0.068	0.946	-0.104	-1.258	0.210
Total TEQ	0.010	0.110	0.912	-0.080	-0.957	0.340

ND：non-detectable，

Multiple regression. The concentrations of PCBs and Dioxins were analyzed with log transformation. Adjusted for gestational days, blood sampling time, and smoking during pregnancy. * $p < 0.05$; ** $p < 0.01$

The relations between exposure concentrations and effects

- The previous studies in various countries such as Netherlands, Germany, America, etc. utilized only total PCB or TEQ values as exposure indices, --------thus it is difficult to make stringent comparisons with those studies.
- Our current data in Sapporo, Hokkaido shows that psychomotor development is significantly negatively related only to isomers of dioxins. Especially, motor development significantly negatively related to several isomers of dioxins.
- The present finding of no association between PCB and psychological development in child neural development is consistent with previous studies.
- Although several specific isomers of dioxins negatively influences motor development at low-background levels of exposure concentrations, the total levels of PCBs or total TEQ values were not significantly associated with motor development.

Comparison of background-level concentrations of PCBs in maternal blood between previous studies and current study.

Longnecker et al.

PCB153 in the previous studies are from Environ Health Perspect. 2003 Jan;111(1):65-70

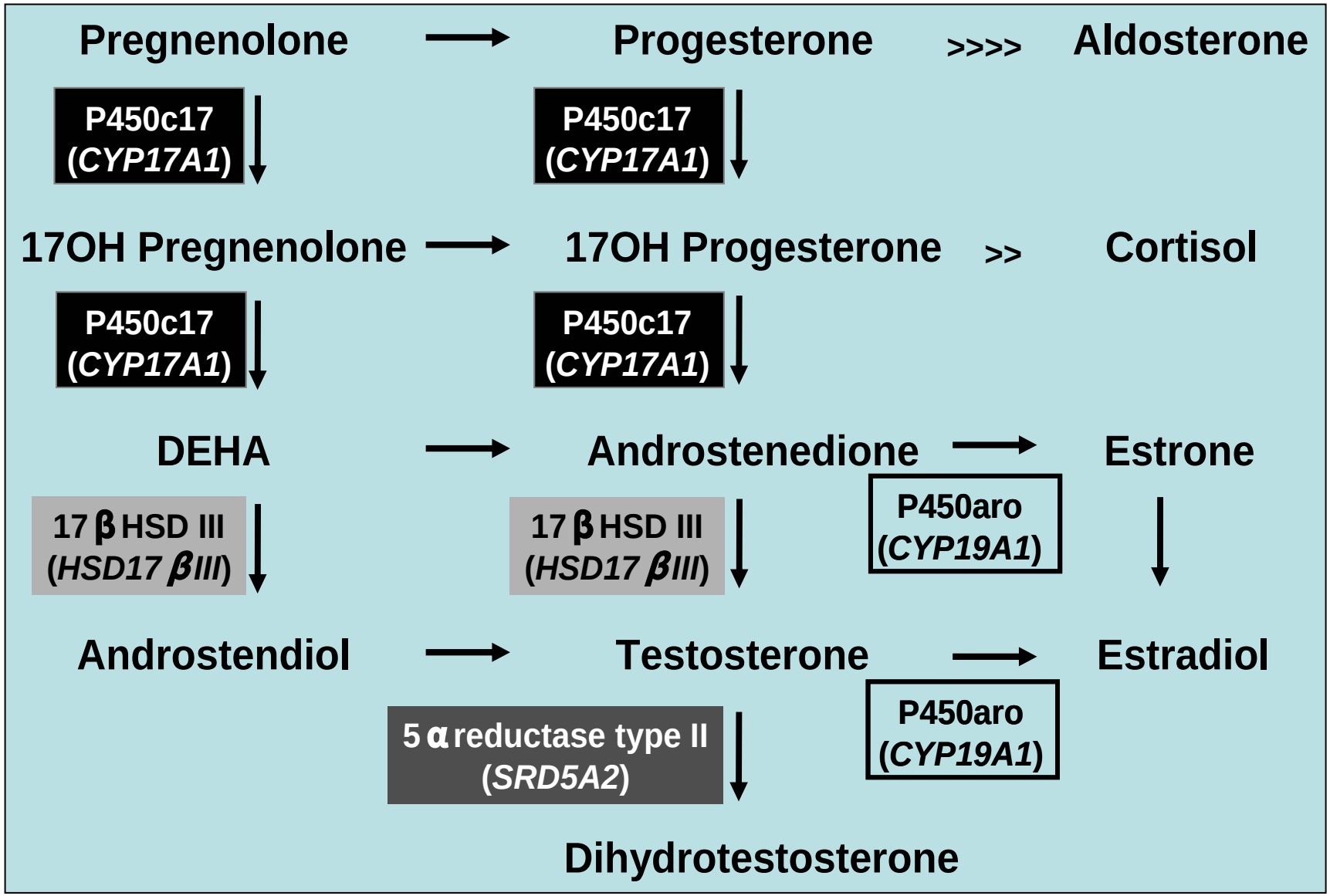
Regions	Sampling period	A number of samples	PCB 153 Median (ng/g lipid)
U.S.A/ 11 regions	1959-1965	2737	140
U.S.A/North Carolina	1978-1982	872	80
Netherlands/ 2 regions	1990-1992	415	100
Germany /Düsseldorf	1993-1995	126	140
Current study/Sapporo	2002-2004	64	22.9

Exposure level in Sapporo is lower than those in Germany, Netherlands, and U.S.A/North Carolina and about same as those in U.S.A./New York and U.S.A./Massachusetts. Using the data from Iida et al. (1999), Wang reported that TEQ level in Japan was same as that in Korea in 2002 and nearly same as that in U.S.A. .

Genetic Susceptibility Factors

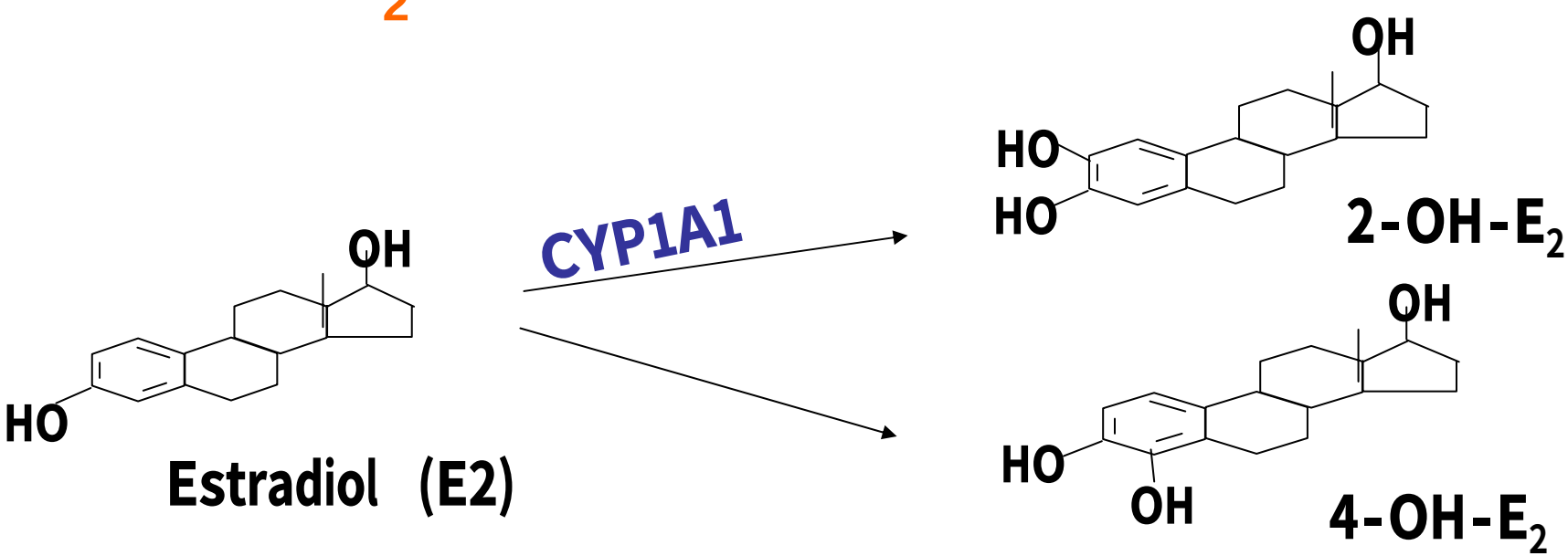
1. Polymorphisms in genes associated with metabolism of environmental chemicals
2. Polymorphisms in genes of disease etiology (eg., hormone synthesis)
3. Maternal (Paternal) vs. Child

Steroid Synthesis



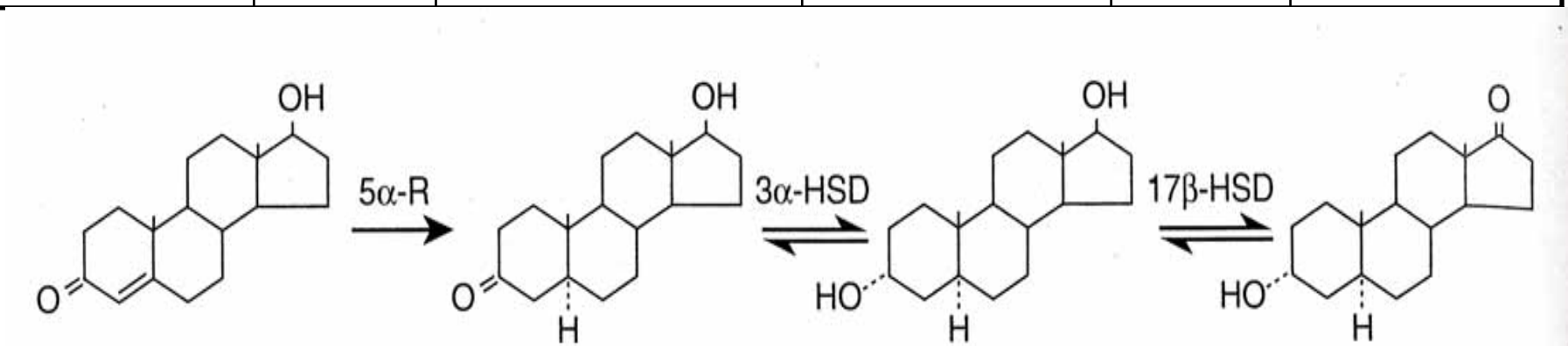
Maternal genetic polymorphisms and the risk of hypospadias

		Case (%)	Control (%)	Adjusted OR	95%CI
GSTM1	Present	41.9	49.2		
	Null	58.1	50.8	1.11	0.34-3.64
GSTT1	Present	61.3	54.2		
	Null	38.7	45.8	1.14	0.34-3.79
CYP1A1	m1/m1	48.4	28.1		
	m1/m2+m2/m2	51.6	71.9	0.28	0.08-0.97



Infant genetic polymorphisms and the risk of hypospadias

		Severe Case (%)	Control (%)	OR	95%CI
SRD5A2 (V89L)	G/G	10.0	23.8		
	C/C	23.3	11.9	4.67	1.02-21.33
		Mild Case (%)	Control (%)	OR	95%CI
HSD17B3 (A39G)	G/G	23.3	35.6		
	S/S	30.2	16.8	2.75	1.01-7.53



Testosterone

Dihydrotestosterone

3α-Androstanediol

Androsterone

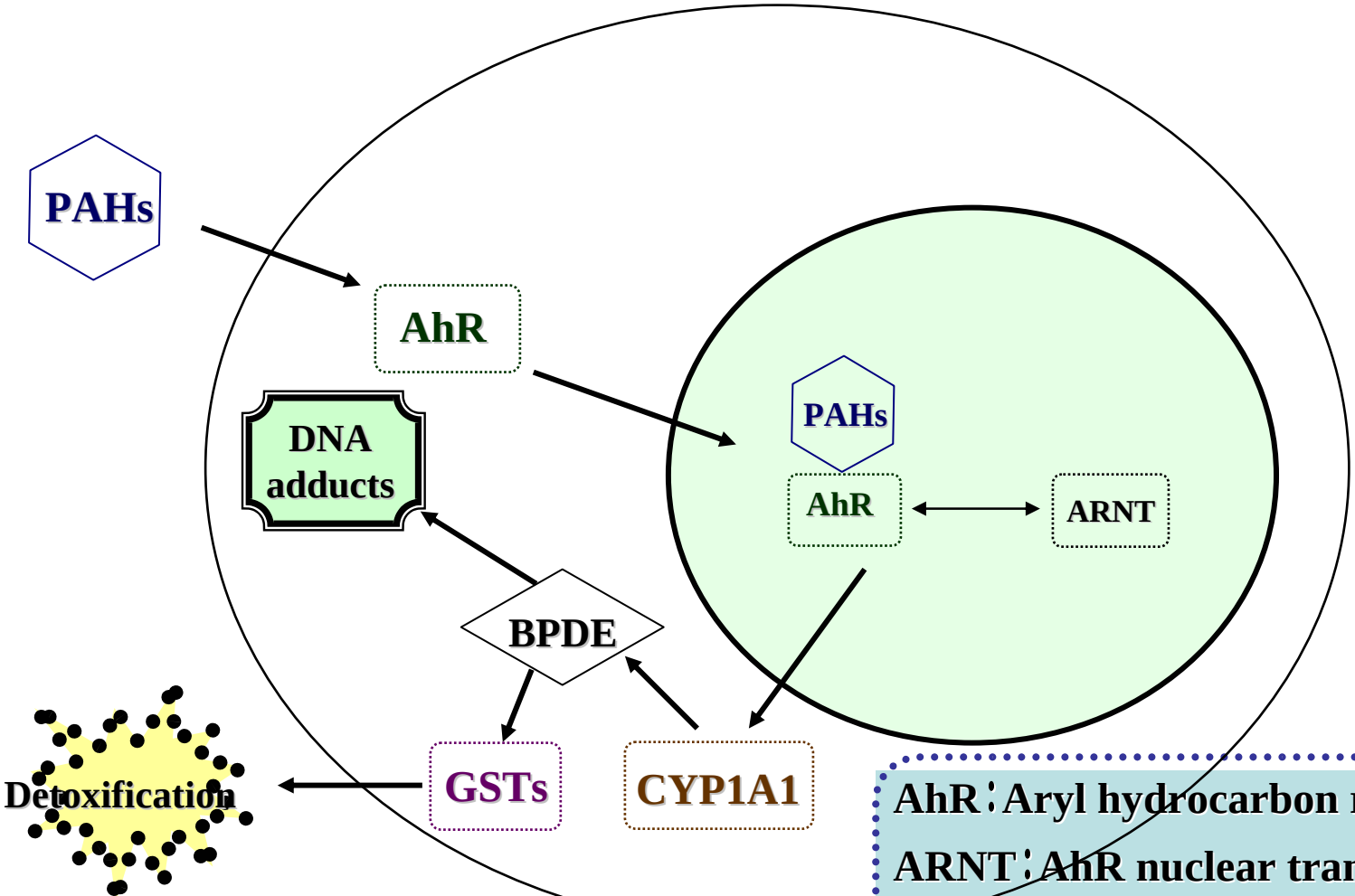
Environmental factors, which might strongly confound with EDCs

Maternal Smoking

Well-known Adverse Health Effects;

- **Pregnancy outcomes**
- **Birth outcomes**
  - **Low birth weight/height**
  - **Small head circumference**
- **Allergy**
- **Developments**

Metabolites of polycyclic aromatic hydrocarbons (PAHs)



AhR：Aryl hydrocarbon receptor

ARNT：AhR nuclear translocator

CYP：Cytochrome P450

GSTs：Glutathione-S-transferases

BPDE：B[a]p-7,8-dihydrodiol-9,10-epoxide

Reference：Baccarelli *et al*. (2004)，Rybicki *et al*. (2005)

Hokkaido study of Children and environment (Summary)

1. Conduct prospective cohort studies rather than retrospective case-control studies
2. Follow them up as long as possible
3. Carefully adjust for all confounding variables in each period and for each outcome.
4. Take into account total environment, not only EDCs, but also maternal consumption of folic acid/iodine during pregnancy, breast milk, parental smoking, postnatal social/home environment etc.
5. Consider individual susceptibilities both of child and parents, especially mother's.

For the further study ①

1. Prenatal environmental factors might have possible negative effects on low birth weight and congenital anomalies.
2. Exposure to dioxins and PCBs might influence neurobehavioral development during infancy and childhood.
3. It is needed to consider about additive effects of various chemicals which surround our daily lives (e.g. mercury, pesticide, etc.).
4. It is possible that nutrition during pregnancy (eg. folic acid), smoking, breast-feeding, and a good home environment would improve (or modify and antagonize) the influence.
5. There may be a high risk group by genetic susceptibility factors.

For the further study ②

6. The effects of various chemicals that are frequently used around the world such as PFOS and phthalates as well as dioxins and PCBs should be also evaluated.

7. The environmental factors such as diets of infants and children and overall air quality should be comprehensively examined.

8. Genetic susceptibility factors should not be ignored.

   Genetic factors for chemical metabolites

   Genetic factors for diseases

9. Follow up the development of children and to do longitudinal observation for a long-term period.

10. Governmental Supports are necessary.

Dept. of Public Health Sciences, Hokkaido University School of Medicine

Fumihiro Sata, Yasuaki Saijo, Norie Kurahashi, Shizue Kato, Sonomi Nakajima, Seiko Sasaki, Tomoko Kondo, Akiko Uno, Susumu Ban, Washino Noriaki, Konishi Kanae, Toh Tomoko, Kana Suzuki, Shigeyuki Matsuzawa

Toho Obstetrics & Gynecology hospital (札幌東豊病院)

Hokkaiodo Association of Obstetricians and gynecologists (40 institutes (北海道産婦人科医会)

We sincerely thank that great number of mothers and children in the regions could participate in our research.

Collaborating Institutions

Hokkaido University School of Medicine
Department of Urology
Department of Perinatal Medicine
Department of gynecology

Hokkaido University Graduate School of Veterinary Medicine
Department of Environmental veterinary Sciences

Sapporo Medical University
Department of Obstetrics and Gynecology

Toho Obstetrics & Gynecology hospital

Sapporo city Institute of Public Health

Asahikawa Medical College
Department of Obstetrics & Gynecology

Hokkaido Association of Obstetricians and gynecologists
(40 institutes)

Fukuoka Institute of Health and Environmental Science

Hoshi University
Department of Analytical Chemistry