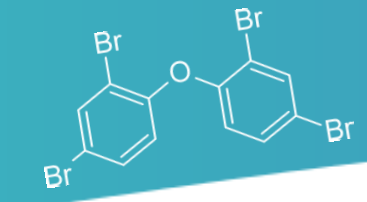

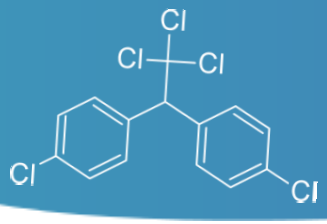

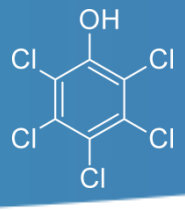

Is the contamination of the Belgian population by endocrine disruptors linked to thyroid disorders ?

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LIÈGE université

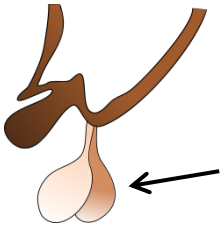
CHU de Liège

CIRM

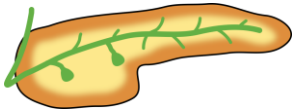
Endocrine system

A **hormone** is a molecule produced by an endocrine gland that travels through the blood to produce effects on distant cells and tissues (Melmed & Williams, 2011)

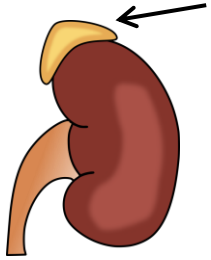
The main **endocrine glands** are:



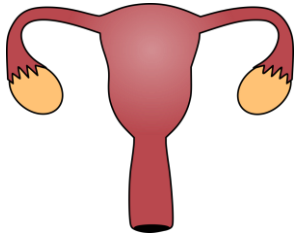
Pituitary gland
Growth hormone, prolactine,…



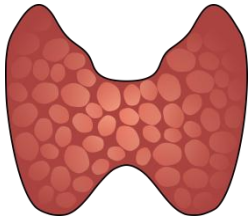
Pancreas
Insulin, glucagon



Adrenal glands
Cortisol, epinephrine,…

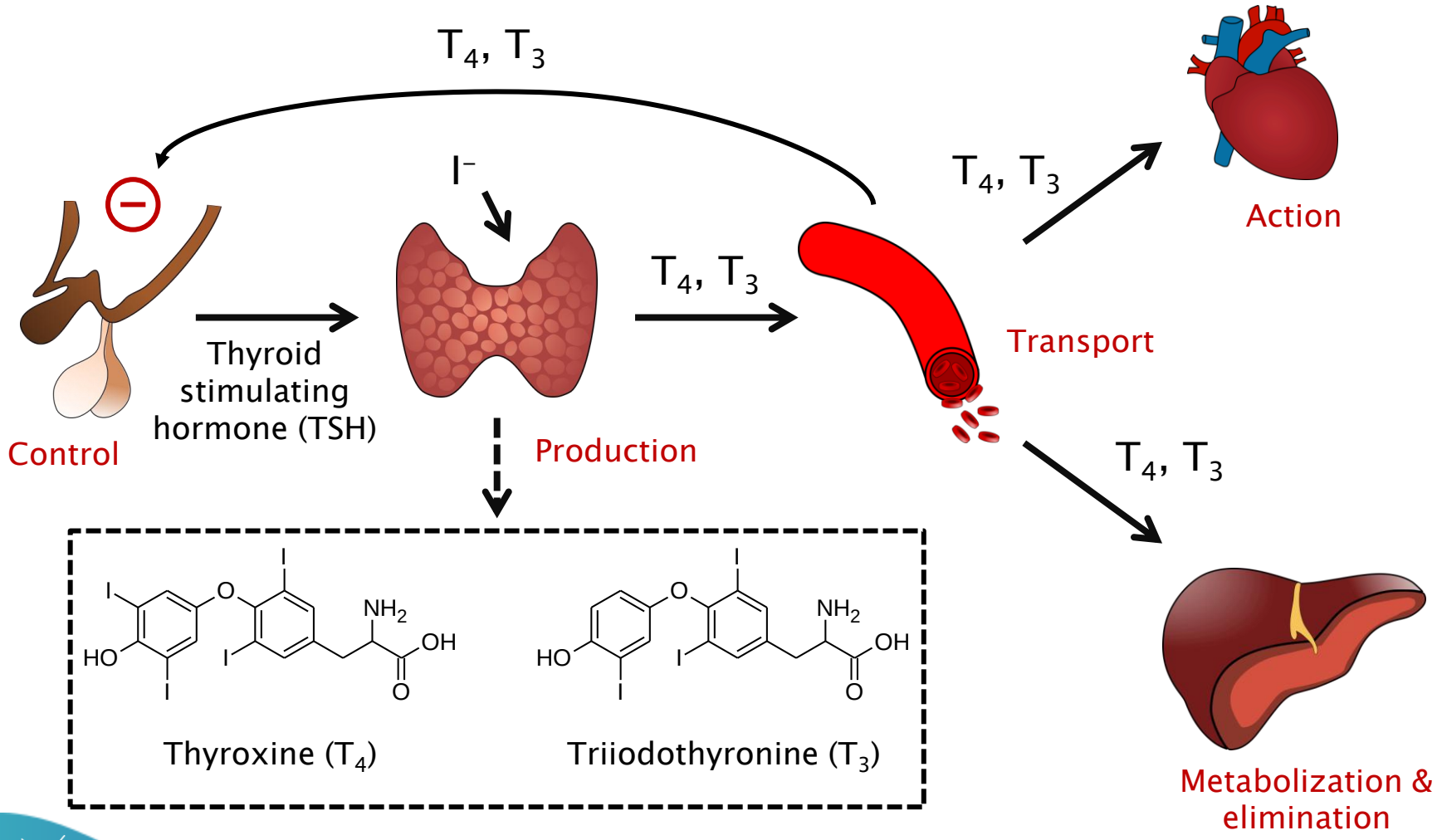


Gonads
Estrogens, androgens,…

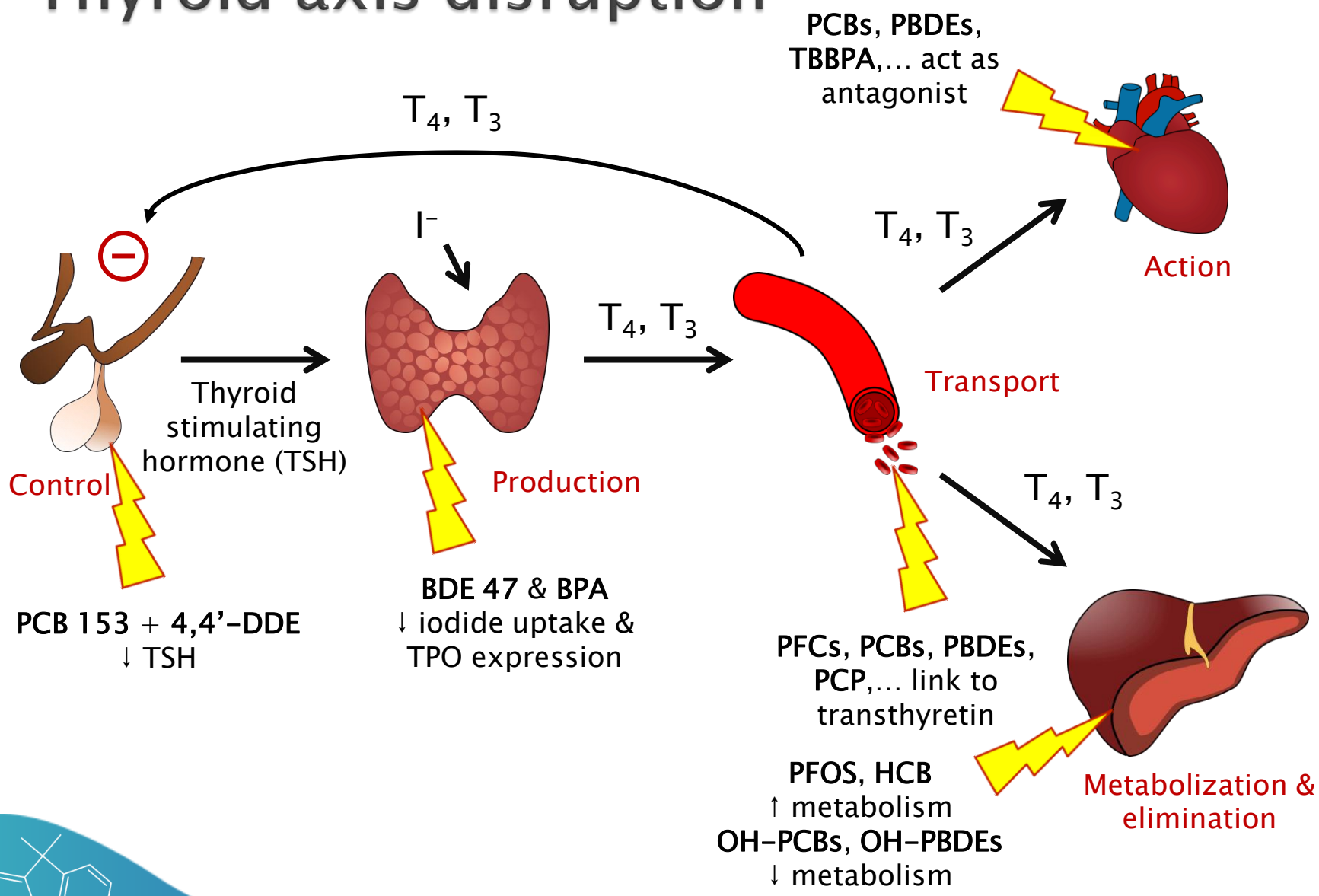


Thyroid
Thyroid hormones

Thyroid axis

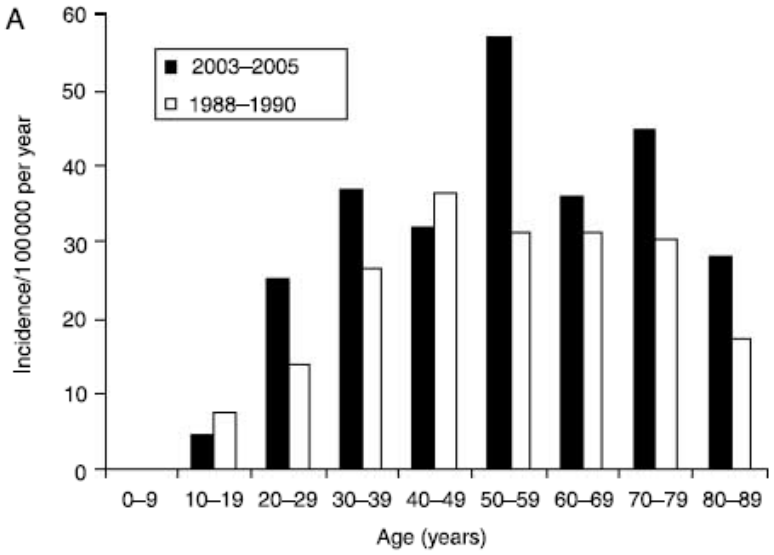


Thyroid axis disruption



Major public health concern

- Disruption may lead to hyper- or hypothyroidism, two pathologies for which the incidence is growing.



Age-specific incidence of Basedow hyperthyroiditis in Malmö (Sweden) (Lantz, 2009)

Major public health concern

- Critical during the pregnancy and the early childhood (neurodevelopment)
- Mother or congenital hypothyroidism leads to cretinism

- In healthy pregnant women, even a slight reduction of the thyroid hormones levels is associated with lower IQ score in the children

Objective

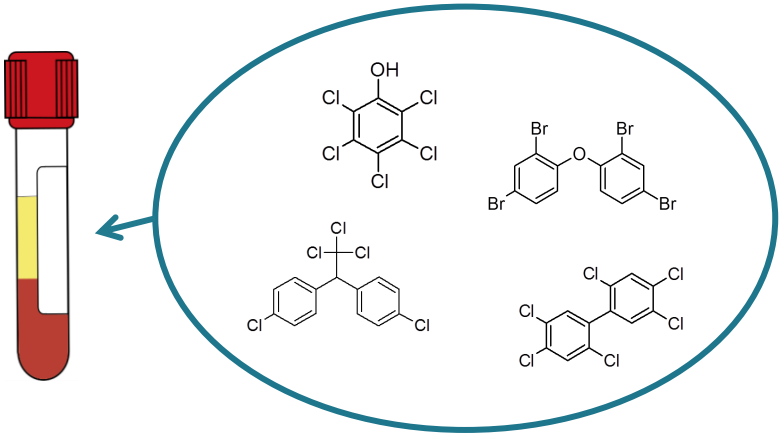
1. Thyroid disruptors exist
2. We are exposed
3. Health consequences

BUT

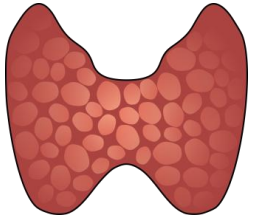
1. High concentration
2. *In vitro* & animal models ≠ Human
3. Few epidemiological studies

- The objective of our work is to contribute to the assessment of the potential disruption of the human thyroid system by some environmental pollutants.

To reach this objective:



Assessment of the contamination in Belgian populations



Assessment of thyroid function

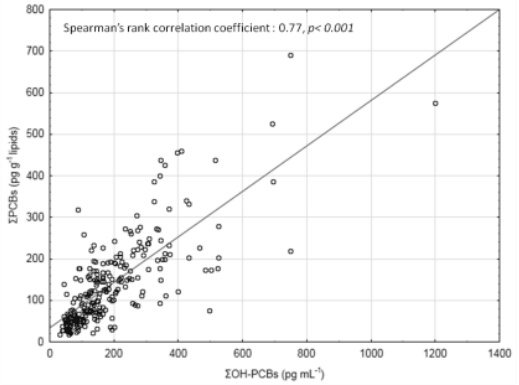


Figure 2: correlation between the concentrations of ΣOH-PCB and ΣPCBs.

Statistical analyses to evaluate the associations between the parameters

Two axes:

- Newborns

- Adults with thyroid pathologies

Newborns

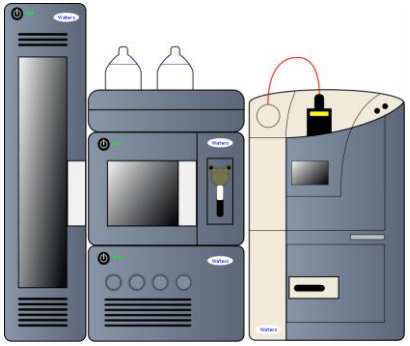
- We collected 281 cord blood samples (52.8% ♂ & 47.2% ♀)
- Obstetric service of the University Hospital of Liege
- Collected between 2013 and 2016
- Data:
  - TSH (neonatal screening test)
  - Sex
  - Birth weight
  - Mother age
  - Gestational age
  - Pre-pregnancy BMI
  - Parity
  - Tobacco habits
  - Hypothyroidism in the mother

Perfluoroalkyl substances

193×

Solid phase extraction (Oasis WAX)

LC–MS/MS

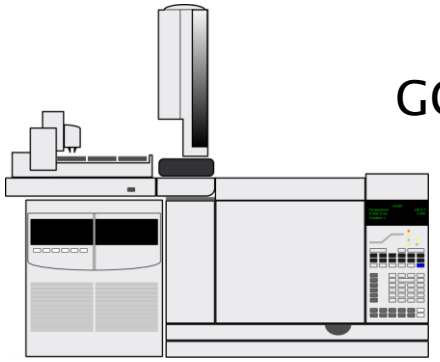


Organochlorine pesticides and PCBs

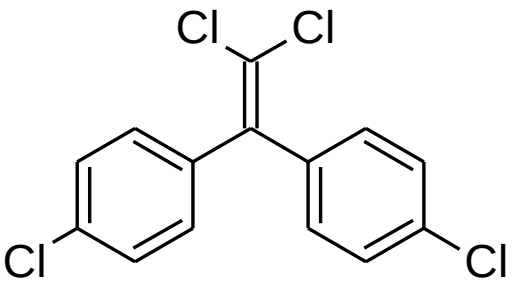
191×

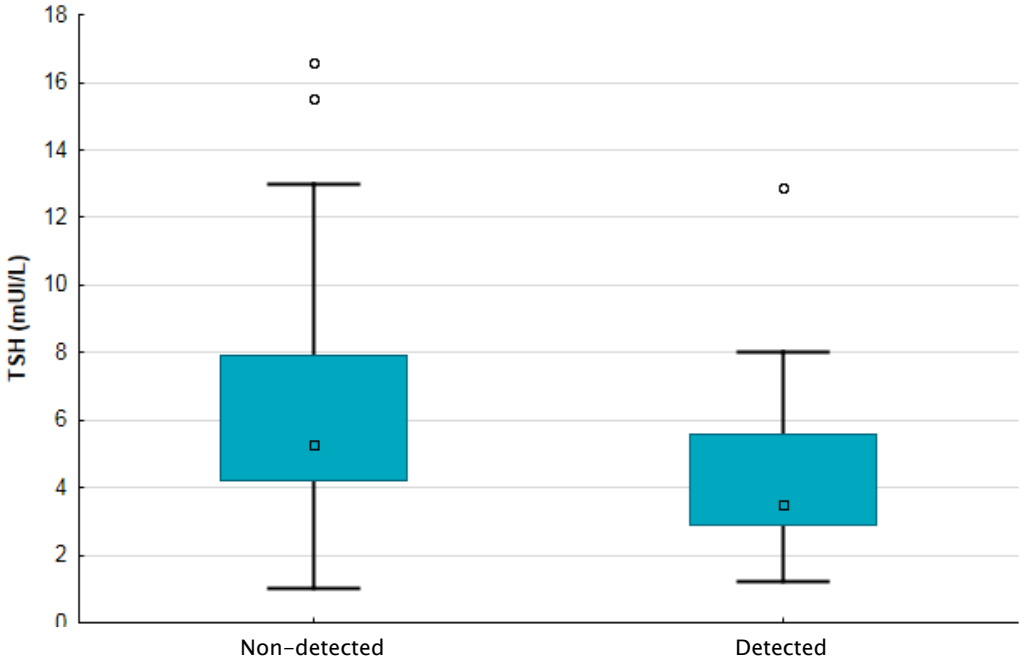
Solid phase extraction (Bond Elut Certify)

GC–MS



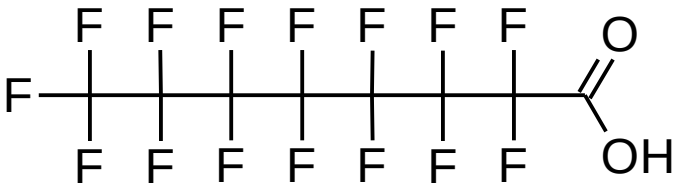
- In male newborns with detectable levels of 4,4'-DDE, there is an significant diminution of TSH levels compared to newborns with no detectable levels of 4,4'-DDE (p=0,035).

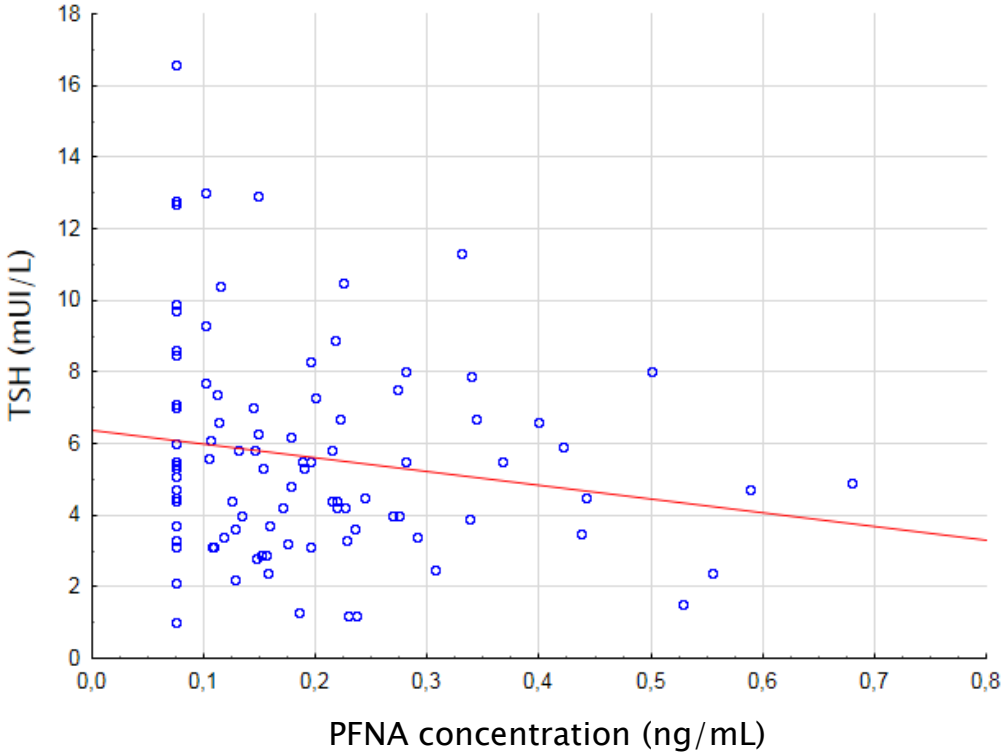


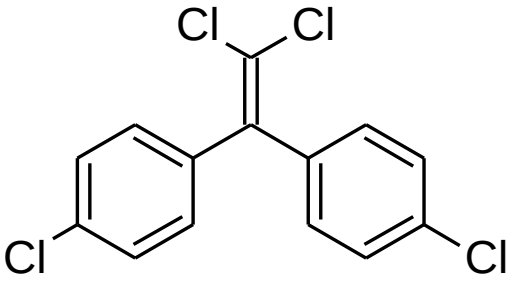


4,4'-DDE, ND vs D
in male newborns

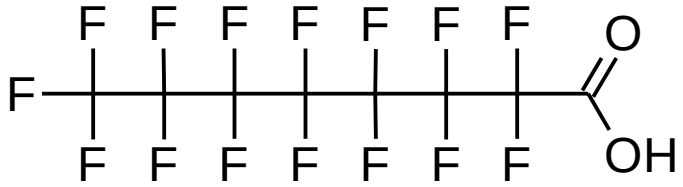
- Negative correlation between PFNA levels and TSH levels in male newborns (p=0,01).



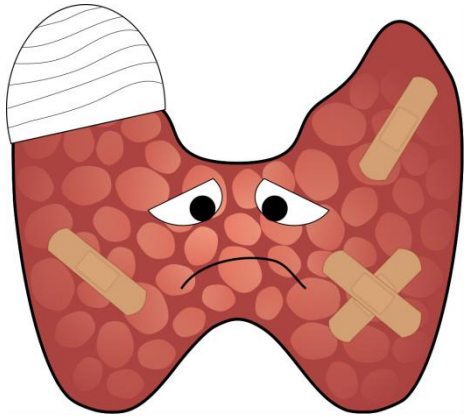




4,4'-DDE

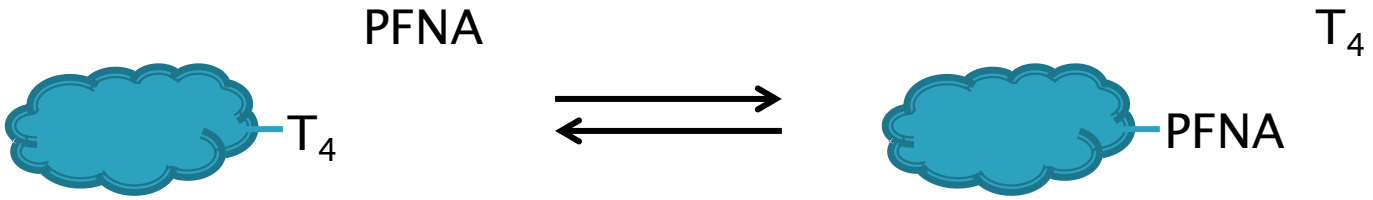


PFNA

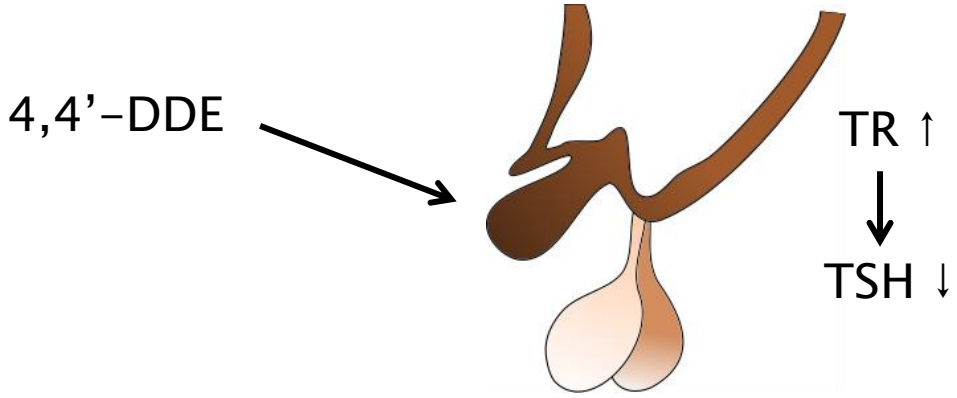


Consequences on neurodevelopment ?

- Some mechanisms may explain our results:
  - PFNA displace T_4 from blood transport proteins ➔ free T_4 ↑ & TSH ↓ (negative feedback)



  - In rat, 4,4'-DDE disrupts the hypothalamus-pituitary axis which consequently reduces the TSH level



- We must be careful when we interpret the results of <u>one single</u> epidemiological study.
  - Inverse causality?
  - Missed confounding factor?
  - Statistical power?
  - …
- Results under revision in Environmental Pollution

Contents lists available at ScienceDirect

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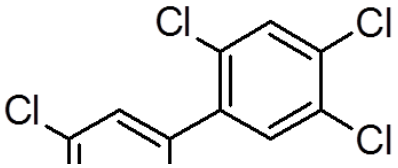
journal homepage: www.elsevier.com/locate/envpol

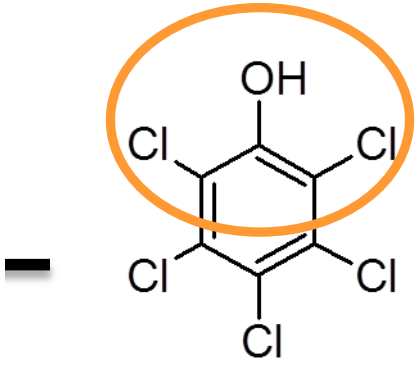
Adults



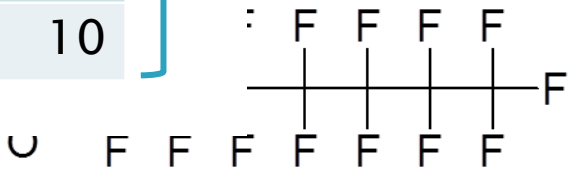
Affinity for transthyretin

Compounds	Relative potency (T_4)
PFOA	0.064
PCB 153	0.55
BDE 47	0.0022
4-OH-CB 107	3.3
6-OH-BDE 47	0.26
PCP	0.6
TBBPA	1.6
2,4,6-TBP	10

POHs



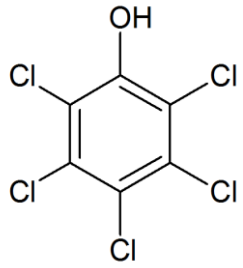
Organohalogenated phenolic compounds ✗



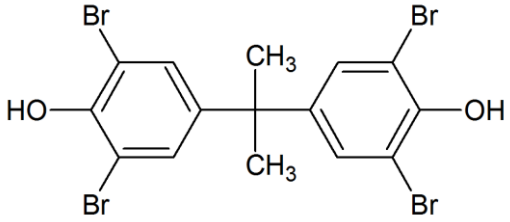
Perfluoroalkyl substances ✓

Organochlorine pesticides ✓

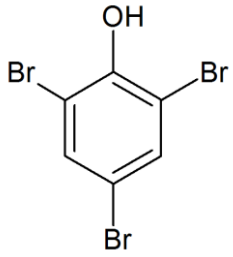
We developed a analytical method to determinate these compounds in serum



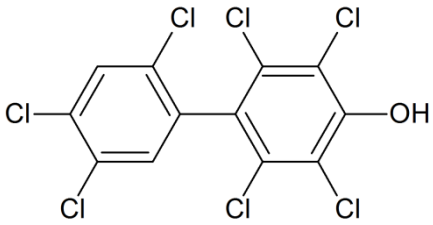
Pentachlorophenol:
pesticide, wood protector



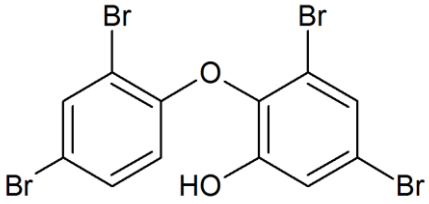
Tetrabromobisphenol A:
brominated flame retardant



5 Bromophenols:
brominated flame retardants, synthesis intermediates, metabolites



7 Hydroxy-PCBs:
PCBs' metabolites



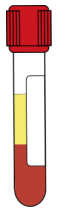
3 Hydroxy-PBDEs:
PBDEs' metabolites

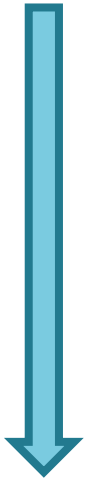
Objective

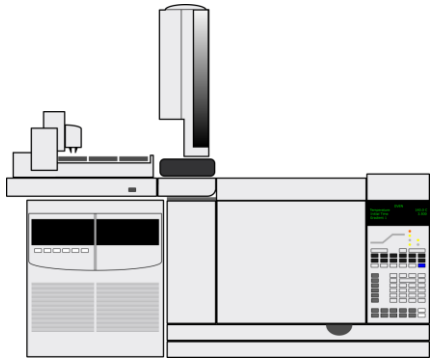
- Apply this method for large epidemiological studies (200-300 samples).
  - Existing methods in the literature BUT numerous time & solvent consuming steps
  - Reach high sensitivity (some pg/mL) (in comparison, paracetamol: 5-25 µg/mL)
  - Use reasonable volume of serum
  - Perform a solid validation

Analytical method

- Sample (1ml serum)
- Denaturation (formic acid)
- SPE (Oasis MAX cartridge)
  - Conditioning cartridge
  - Loading sample
  - Ionizing
  - Washing cartridge
  - Eluting neutral compounds
  - Eluting phenolic compounds
- Liquid-liquid extraction
- Derivatization
- Reconstitute in iso-octane
- GC-MS







- Validation with triplicate validation samples during three days and calibration curve in duplicate

Table 5
Trueness, precision, uncertainty, limit of detection (LOD), lower limit of quantification (LLOQ), upper limit of quantification (ULOQ) and correlation coefficient (R^2) for each analyte.

	Target Conc. (pg mL^{-1})	TBBPA	2,3,6-TBP	2,4,6-TBP	2,4,5-TBP	2,3,4,6-TeBP	4-OH—CB 107	3-OH—CB 138	4-OH—CB 146	3-OH—CB 153	4-OH—CB 172	3-OH—CB 180	4-OH—CB 187	6-OH-BDE 47	5-OH-BDE 47	5′-OH-BDE 99	Target Conc. (pg mL^{-1})	PCP
Trueness	**2**	–	–	–	–	–	–	–	–	–	6.25	–	−5.41	–	–	0.91	**20**	–
Relative bias (%)	**5**	−0.22	0.68	–	12.04	−0.15	−2.74	3.98	−3.72	−1.44	9.03	−1.56	−1.99	4.93	5.76	5.10	**50**	0.83
	10	2.40	9.78	–	11.00	8.30	7.92	8.41	9.51	6.53	10.30	8.13	−2.59	7.28	6.60	9.29	**100**	5.29
	25	4.55	−0.75	–	11.79	2.03	4.36	2.52	6.08	5.16	3.90	1.82	6.96	2.19	3.49	6.63	**250**	7.17
	50	0.07	2.13	2.14	−1.54	−2.00	6.09	2.74	5.37	6.20	4.05	4.13	4.53	−1.66	−0.69	6.32	**500**	12.77
	500	−1.98	–	3.04	–	4.17	5.47	3.82	4.38	6.16	2.35	3.65	0.38	−0.77	−3.25	6.96	**5000**	−13.11
	800	−1.18	–	–	–	3.93	4.69	0.24	0.68	2.35	−0.92	0.10	−2.40	0.07	−0.25	4.60	**8000**	−11.86
Intra assay precision	**2**	–	–	–	–	–	–	–	–	–	11.97	–	6.24	–	–	13.21	**20**	–
Repeatability (RSD%)	**5**	15.77	10.93	–	17.07	15.92	9.65	11.44	9.97	8.54	4.63	4.40	4.07	6.35	7.90	10.77	**50**	8.13
	10	15.48	9.70	–	8.76	6.25	5.57	4.01	6.16	8.39	3.36	7.01	2.36	4.83	5.68	7.91	**100**	10.10
	25	12.75	8.72	–	8.24	7.78	7.16	5.43	5.08	3.17	2.35	1.77	6.57	6.70	6.12	3.05	**250**	7.13
	50	3.52	15.03	11.83	12.51	13.60	5.37	4.02	4.47	4.00	2.99	3.20	6.97	4.31	5.46	4.38	**500**	3.31
	500	9.18	–	8.55	–	6.47	2.87	2.04	2.03	2.52	1.37	1.63	1.06	4.08	4.69	4.83	**5000**	1.44
	800	11.96	–	–	–	9.05	4.67	4.19	3.56	4.27	2.65	2.82	2.60	8.68	10.34	6.79	**8000**	3.55
Inter assay precision	**2**	–	–	–	–	–	–	–	–	–	13.22	–	15.99	–	–	16.06	**20**	–
Intermediate precision (RSD%)	**5**	15.77	17.88	–	17.68	21.29	16.83	13.37	11.39	16.76	6.41	5.87	7.63	9.40	10.93	11.80	**50**	11.10
	10	18.15	11.12	–	9.10	10.95	14.83	7.66	11.19	13.89	3.67	7.01	4.45	9.24	9.51	10.96	**100**	10.10
	25	12.75	15.19	–	9.57	9.19	12.13	10.58	8.39	9.27	3.44	2.86	11.34	7.30	8.49	4.68	**250**	7.13
	50	15.27	17.55	15.88	13.59	16.57	12.04	9.31	9.20	11.67	3.22	4.19	6.97	12.44	15.42	9.18	**500**	4.21
	500	15.00	–	13.78	–	12.08	6.55	11.83	10.99	11.36	1.37	3.15	2.93	13.06	7.84	10.78	**5000**	8.14
	800	14.68	–	–	–	13.13	11.28	9.10	8.82	8.84	3.43	3.69	4.48	13.70	16.91	10.17	**8000**	7.89
Uncertainty	**2**	–	–	–	–	–	–	–	–	–	27.89	–	36.48	–	–	34.26	**20**	–
Relative expanded uncertainty (%)	**5**	33.09	39.01	–	37.52	45.98	36.87	28.41	24.56	36.88	13.82	12.62	17.20	20.37	23.48	24.88	**50**	24.45
	10	38.77	23.58	–	19.19	23.94	32.86	16.82	24.52	30.30	7.73	14.58	10.02	20.37	20.76	23.63	**100**	21.29
	25	27.26	33.21	–	20.32	19.53	26.48	23.24	18.30	20.58	7.45	6.23	24.81	15.38	18.31	10.17	**250**	15.04
	50	34.06	37.27	34.41	28.63	35.34	26.57	20.56	20.25	25.90	6.76	9.00	24.51	27.58	34.20	20.21	**500**	9.03
	500	32.70	–	30.08	–	26.49	14.89	26.41	24.52	25.32	2.84	6.90	6.49	29.02	17.56	23.80	**5000**	18.15
	800	31.42	–	–	–	28.51	24.97	20.07	19.74	19.46	7.37	7.90	9.81	29.83	36.90	22.08	**8000**	17.42
LOD (pg mL^{-1})		1.24	0.71	15.00	1.51	1.24	0.96	0.92	0.67	0.90	0.67	0.67	0.67	0.76	0.70	0.67		13.50
LLOQ (pg mL^{-1})		4.10	2.35	49.60	5.00	4.10	3.16	3.05	2.20	2.98	2.00	2.07	2.00	2.50	2.31	2.00		44.55
ULOQ (pg mL^{-1})		800	195	722	135	800	800	800	800	800	800	800	800	800	800	800		8000
R^2		0.991	0.9821	0.9864	0.9942	0.9996	0.9999	0.9999	0.9998	0.9998	0.9999	0.9999	0.9999	0.9998	0.9990	0.9994		0.9984

- Number of steps ↓ ✓
- Sensitivity: LOQ between 2 and 5 pg/mL for all the compounds except for PCP: 45 pg/mL and 2,4,6-TBP: 50 pg/mL ✓
- Reasonable volume of serum (1 mL) ✓
- Validated for 16/17 compounds ✓

This method was published:

Journal of Chromatography B, 1036 (2016) 66–75

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Validation of a novel and rapid method for the simultaneous determination of some phenolic organohalogens in human serum by GC–MS

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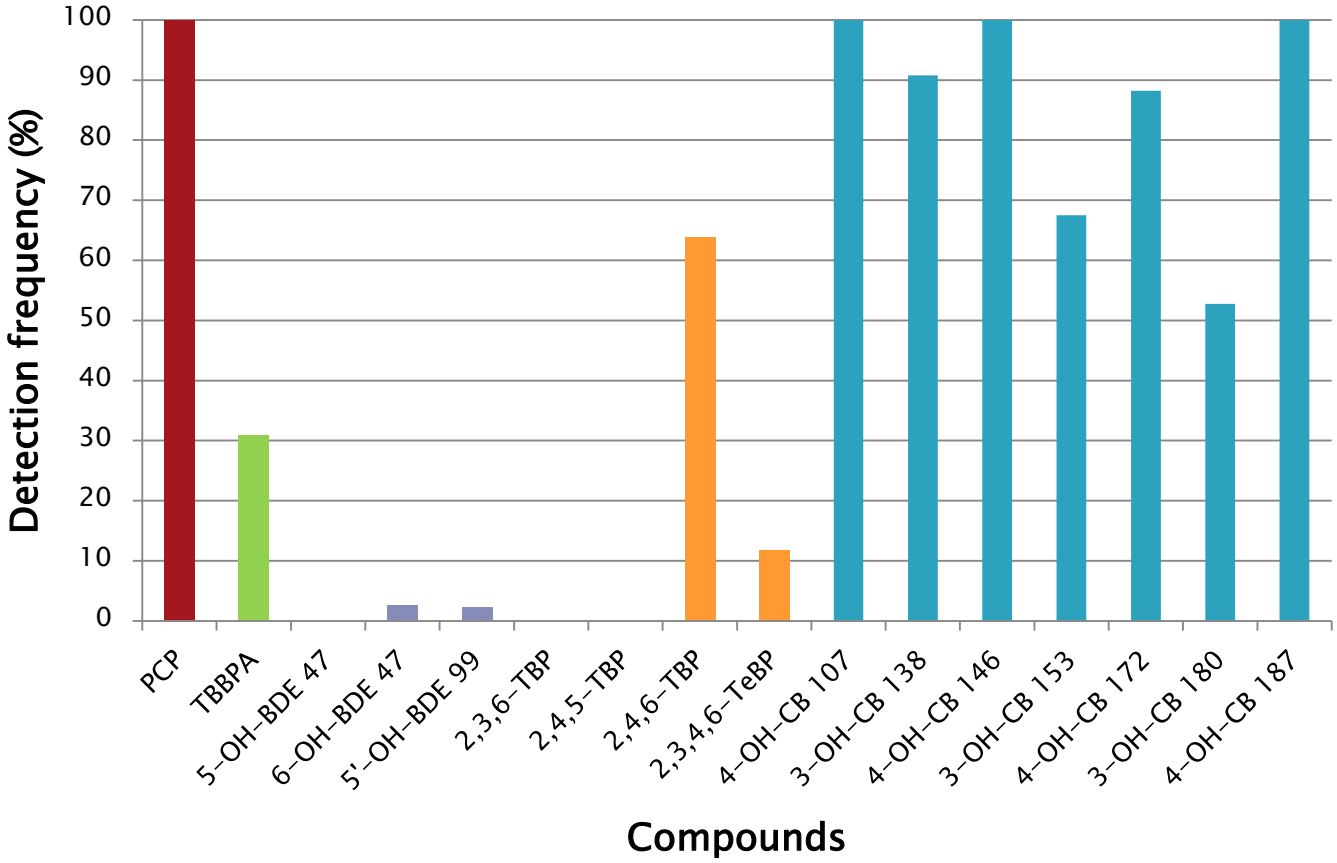
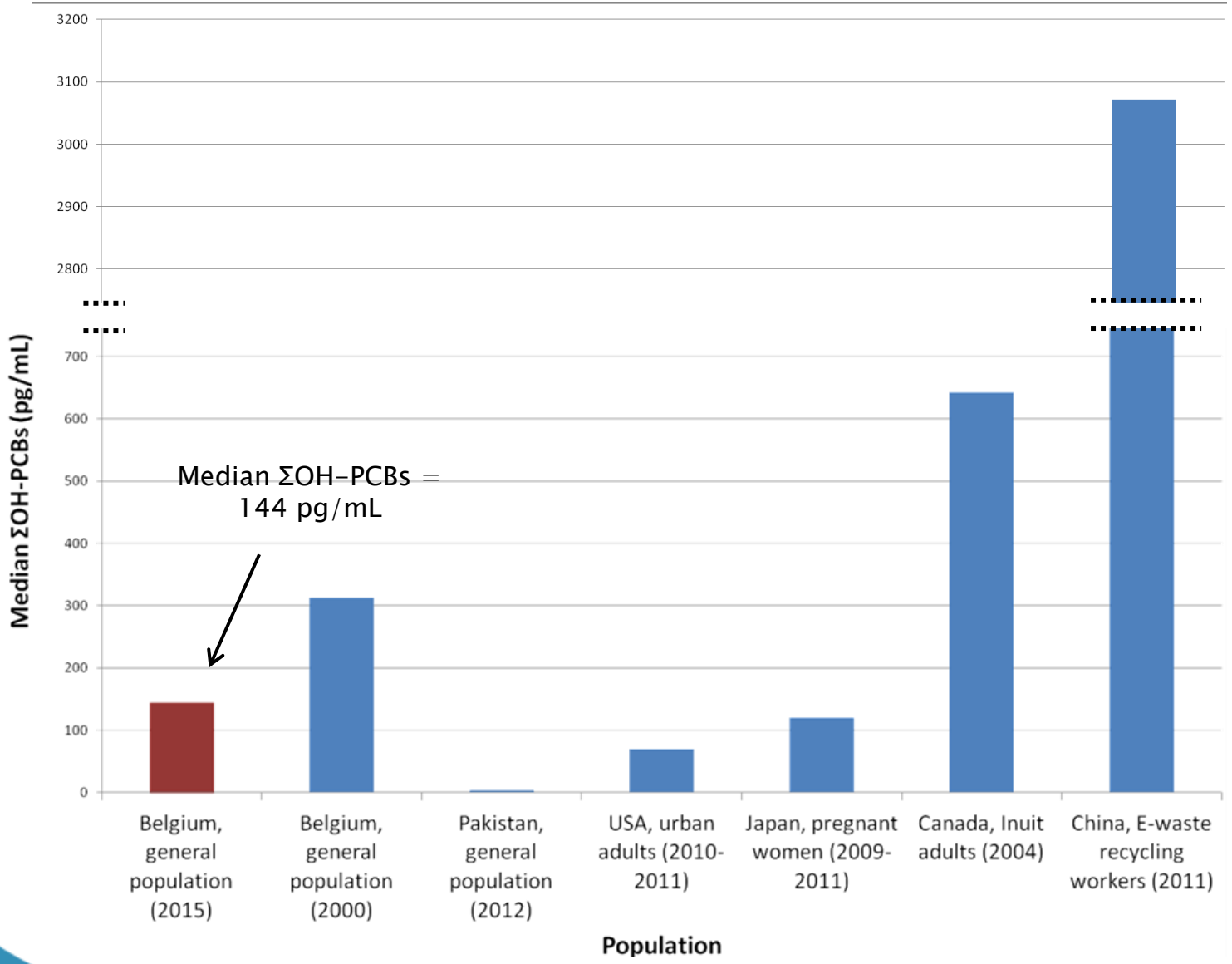
- We applied this method on 272 samples collected in the Province of Liège



Comparison with other populations



These results are published:

Science of the Total Environment 599–600 (2017) 1856–1866

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Determination of phenolic organohalogens in human serum from a Belgian population and assessment of parameters affecting the human contamination

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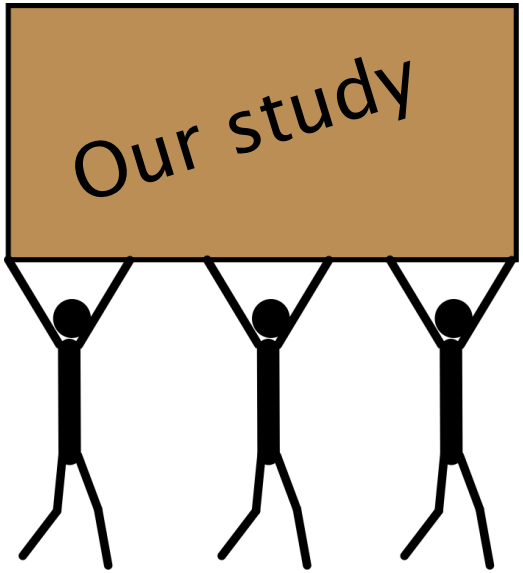
Thyroid pathologies in adults

- Recruit **50 hypothyroid** patients (Hashimoto) & **50 hyperthyroid** patients (Basedow).
- Determinate in serum, **contamination** by PCBs, organochlorine pesticides, perfluoroalkyl substances, brominated flame retardants & phenolic organohalogenated compounds.
- **Compare** contamination with **healthy population**.

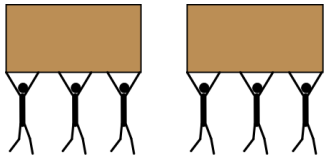
IN PROGRESS

Conclusions

- It doesn't exist a miracle study that will prove with no doubt, that this or that chemical is a thyroid disruptor in human at environmental concentration.
- Our works are important because it constitutes another brick to add to the weight of evidence.



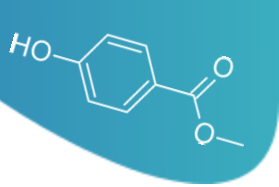
- By adding our work to previous and future studies, maybe that the link between pollution and thyroid function will be finally firmly established.



Global conclusions

- Since many years, we develop expertise to assess the contamination by several pollutants in human
- Assessment of the contamination in the Belgian general population
- Impact on thyroid function

- New chemicals: Bisphenol A alternatives
- Other pathologies: Diabetes



Thank you for your attention