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

P. Dufour, C. Pirard, C. Charlier
Laboratory of Clinical, Forensic and Environmental Toxicology, University of Liege (ULiège)
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

Control

Thyroid stimulating hormone (TSH)

Production

\( T_4, T_3 \)

Action

Transport

\( T_4, T_3 \)

Metabolization & elimination

Thyroxine (T4)

Triiodothyronine (T3)
Thyroid axis disruption

Thyroid stimulating hormone (TSH)

Control

PCB 153 + 4,4’-DDE ↓ TSH

Thyroid

Production

BDE 47 & BPA ↓ iodide uptake & TPO expression

Transport

T4, T3

Action

T4, T3

PCBs, PBDEs, TBBPA,… act as antagonist

Metabolization & elimination

PFOS, HCB ↑ metabolism

OH-PCBs, OH-PBDEs ↓ metabolism

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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

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

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Perfluoroalkyl substances

Solid phase extraction (Oasis WAX)

193×

LC–MS/MS

Organochlorine pesticides and PCBs

Solid phase extraction (Bond Elut Certify)

191×

GC–MS

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In male newborns with detectable levels of 4,4’-DDE, there is a significant diminution of TSH levels compared to newborns with no detectable levels of 4,4’-DDE ($p=0.035$).
Negative correlation between PFNA levels and TSH levels in male newborns ($p=0.01$).
Consequences on neurodevelopment?
Some mechanisms may explain our results:

- PFNA displace T\textsubscript{4} from blood transport proteins \rightarrow free T\textsubscript{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 one single epidemiological study.

- Inverse causality?
- Missed confounding factor?
- Statistical power?
- ...

Results under revision in Environmental Pollution
## Adults

### Affinity for transthyretin

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Relative potency (T&lt;sub&gt;4&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFOA</td>
<td>0.064</td>
</tr>
<tr>
<td>PCB 153</td>
<td>0.55</td>
</tr>
<tr>
<td>BDE 47</td>
<td>0.0022</td>
</tr>
<tr>
<td>4-OH-CB 107</td>
<td>3.3</td>
</tr>
<tr>
<td>6-OH-BDE 47</td>
<td>0.26</td>
</tr>
<tr>
<td>PCP</td>
<td>0.6</td>
</tr>
<tr>
<td>TBBPA</td>
<td>1.6</td>
</tr>
<tr>
<td>2,4,6-TBP</td>
<td>10</td>
</tr>
</tbody>
</table>

Organohalogenated phenolic compounds 🗿

POHs

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

<table>
<thead>
<tr>
<th>Step</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample (1ml serum)</td>
</tr>
<tr>
<td>Denaturation (formic acid)</td>
</tr>
<tr>
<td>SPE (Oasis MAX cartridge)</td>
</tr>
<tr>
<td>Conditioning cartridge</td>
</tr>
<tr>
<td>Loading sample</td>
</tr>
<tr>
<td>Ionizing</td>
</tr>
<tr>
<td>Washing cartridge</td>
</tr>
<tr>
<td>Eluting neutral compounds</td>
</tr>
<tr>
<td>Eluting phenolic compounds</td>
</tr>
<tr>
<td>Liquid–liquid extraction</td>
</tr>
<tr>
<td>Derivatization</td>
</tr>
<tr>
<td>Reconstitute in iso–octane</td>
</tr>
<tr>
<td>GC–MS</td>
</tr>
</tbody>
</table>

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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$^{-1}$) | TBP-FA | 2,3,6-TTP | 2,4,6-TTP | 2,4,5-TTP | 2,3,4,6-TeBP | 4-OH-CE 107 | 3-OH-CE 138 | 4-OH-CE 146 | 4-OH-CE 153 | 4-OH-CE 172 | 4-OH-CE 180 | 5-OH-BDE 47 | 6-OH-BDE 47 | 5-OH-BDE 99 | PCP |
|-----------------------------|--------|-----------|-----------|-----------|--------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-----|
| Trueness                    |        |           |           |           |              |             |             |             |             |             |             |             |             |             |     | 20 |
| Relative bias (%)           |        |           |           |           |              |             |             |             |             |             |             |             |             |             |     |    |
| Intra assay precision       |        |           |           |           |              |             |             |             |             |             |             |             |             |             |     |    |
| Repeatability (RSD%)        |        |           |           |           |              |             |             |             |             |             |             |             |             |             |     |    |
| Intermediate precision (RSD%)|        |           |           |           |              |             |             |             |             |             |             |             |             |             |     |    |
| Uncertainty                 |        |           |           |           |              |             |             |             |             |             |             |             |             |             |     |    |
| Relative expanded uncertainty (%)| |       |           |           |              |             |             |             |             |             |             |             |             |             |     |    |
| LOD (pg mL$^{-1}$)          | 0.10   | 0.092    | 0.096     | 0.090     | 0.090       | 0.090       | 0.090       | 0.090       | 0.090       | 0.090       | 0.090       | 0.090       | 0.090       | 0.090       | 0.090 | 0.0908 |
- 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 ✓
Validation of a novel and rapid method for the simultaneous determination of some phenolic organohalogens in human serum by GC–MS

Patrice Dufour, Catherine Pirard, Corinne Charlier

Laboratory of Clinical, Forensic and Environmental Toxicology, University of Liege (ULg), CHU (B35), 4000 Liege, Belgium
Center for Interdisciplinary Research on Medicines (C.I.R.M.), University of Liege (ULg), CHU (B35), 4000 Liege, Belgium
We applied this method on 272 samples collected in the Province of Liège
Comparison with other populations

Median ΣOH-PCBs = 144 pg/mL
These results are published:

**Science of the Total Environment**

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

Patrice Dufour *, Catherine Pirard, Corinne Charlier

Laboratory of Clinical, Forensic and Environmental Toxicology, University of Liege (ULg) CHU (B35), 4000, Liege, Belgium
Center for Interdisciplinary Research on Medicines (CIRM), University of Liege (ULg) CHU (B35), 4000, Liege, Belgium
Thyroid pathologies in adults

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