RIKILT Institute for Food Safety
Part of Wageningen UR

- Works primarily for:
  - Ministry of Economics
  - NVWA
  - EU, EFSA

- Expertises
  - Chemical analyses
  - Bioassays
  - Toxicological research
  - Advice (with RIVM)

- National Reference laboratory for dioxins and PCBs
Dioxins in the news in 2011

Egg scare shuts 4700 farms in Germany
January 8, 2011.

Dioxin cause in German beet pulp found
Animal feed news
18 Nov 2011

Delhaize withdraws organic eggs
Fri 26/08/2011 - 12:11
Adverse health effects of dioxins

Poisoned with a few mg of TCDD
Dioxin poisoning?

- Yushenko hospitalized in September 2004: poisoned?
- Comment Lotti: unlikely since dioxins have modest toxicity
- Blood sample from Vienna hospital tested in December with CALUX-assay at BDS, Amsterdam: strong response
- Result confirmed by two laboratories, Eurofins and RIKILT: only TCDD, levels around 1 ng TEQ/g serum, or 200 ng/g fat
Health risks dioxins overestimated?

“It’s the new symbol for chemicals the government says aren’t so bad after all.”
Dioxins and PCBs

- Which compounds?
- Sources
- Analysis
- Incidents
- Current levels
- Legislation
Dioxins (PCDD/Fs) and PCBs

7 out of 75

10 out of 135

12 out of 209
Sources of PCBs

- Used as technical mixtures and maybe still present in older equipment
  - Transformers
  - Heat exchange
  - Eg Arochlor, Kanechlor, Clophen
- Also used in certain paints and sealants
  - Flame retardent
Sources of dioxins

- Present as contaminants in e.g.:
  - PCB mixtures
  - Pentachlorophenol
  - 2,4,5-T (agent Orange)
- Kaolinic clay and other clays (ball clay, Mabele clay)
- Formed during incineration of waste (fires)
Burning of household waste, also at lower scale and some fires
Properties of dioxins and planar PCBs

- Mixture of 29 congeners with different toxic potencies (including planar PCBs)
- In test animals toxic at very low doses
- Accumulation in fat; slow metabolism and elimination
Toxicity of TCDD

- Most toxic congener
- Effects in animals
  - Endometriosis in monkeys
  - Neurobehavioural effects in monkeys
  - Immune suppression in offspring rats
  - Decreased sperm count in male offspring rats
- Effects at body burdens of 30-70 ng/kg bw
  - Internal dose better than external dose
- Liver tumours in female rats at higher dose levels
  - Recognized human carcinogen (IARC)
Ah-receptor plays a central role

Physiological role of Ah-receptor and natural “hormone”? 
Mechanism behind effects in rats

- Induction of biotransformation enzymes
  - CYPs 1A1, 1A2, 1B1, GST, UDPGT
  - Increased degradation of thyroid hormones
  - Effects on retinoic acid
  - Role of enzyme induction in adverse effects?

- Most sensitive effect: decreased sperm production in rats exposed in utero (in some studies)
  - Decreased testosterone levels (not in every study)
  - Indicates effects on hormone production/degradation
  - Observed after short-term exposure of fetus
Toxicity of TCDD: hazard characterization

- Effects more related to actual levels in the body (body burden) than to intake levels
  - Effects in animals at body burdens of 30-70 ng/kg
- Extrapolation to safe body burden in humans
  - Use of uncertainty factor of (only) 10 for possible interspecies and inter-individual differences in sensitivity
  - Correction for single high dose exposure of rats to repeated low dose exposure (so, no peak exposure)
- Based on half-lives in humans extrapolated to intake of 14-37 pg/kg bw/day for humans
  - Or 1-4 pg TEQ/kg bw/day with UF of 10
Exposure limit (Tolerable Weekly Intake)

- **TWI SCF**: 14 pg TEQ/kg bw/week (exposure limit)
  - Based on effects on fetus, exposed in utero
  - Aiming at prevention of high body burden
- **JECFA**: TMI of 70 pg TEQ/kg bw/month
- **US-EPA**: RfD of 0.7 pg TEQ/kg bw/day
- Further extension possible, eg TYI?
  - Not necessarily, single high dose gives higher exposure of e.g. fetus
TEF values (Toxic Equivalency Factor)

- Exposure limits apply for sum of dioxins and dioxin-like PCBs
- How to deal with this mixture of congeners with different toxic potencies?
- TEQ-principle:
  - Estimate the toxic potency of every dioxin and dioxin-like PCB in comparison to TCDD
  - TEF TCDD=1
Dose-response curves for dioxins and dl-PCBs

Bovee et al. 1998
Dose-response curves for dioxins and dl-PCBs

lower potency

Bovee et al. 1998
TEQ-principle

- **Requirements**
  - All effects through Ah-receptor
  - Effects are additive
  - Only relatively persistent compounds included
- **In vivo data get heavier weight than in vitro data**
  - Kinetics in the body (absorption, distribution, metabolism, excretion) included
- **Each dioxin and PCB obtains a TEF value**
  - TEF for TCDD: 1
  - Current range: 1-0.00003
- **Regularly evaluated (last time 2006)**
Establishment TEFs

- Broad range of values: TEF is weighted value
- Revised in 2005, but only since 2012 applied for official control: check against limits
- Level of mixture expressed in TEQ:
  - TEQ = \( \sum (\text{congener}_i \text{ level}) \times \text{TEF}_i \)
- Change of TEFs has effect on TEQ levels
  - Last change: about 15% reduction in levels
## Change in TEF-values

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### Calculation TEQ level: Belgian feed ‘99

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**Total TEQ level:** 2883 ng/kg, 548 (70%)
Analysis of dioxins and dioxin-like PCBs
GC/HRMS (reference method)

- GC/HRMS: confirmation
  - detection at pg/g levels
  - removal of fat
  - removal of pesticides
  - removal non-dl PCBs
  - detection with GC/HRMS

- Different columns needed
  - Automated clean-up
  - 13C-labeled standards
Relevant issues confirmatory methods

- Application of upperbound principle ("worst case")
  - Levels of non-detected congeners are assumed to be equal to LOQ
  - Lowerbound: non-detects are zero
  - Upperbound level used for checking compliance

- Measurement uncertainty
  - The measurement uncertainty of the method should be established and levels corrected for this
  - So e.g. 1.1 may be reduced to 1.0 before checking compliance
Effect ub vs lb for samples with low levels

- Upperbound levels are a clear overestimation of the level
- So GC/HRMS levels look OK but are not
Performance criteria

- Initially in Commission Directives 2002/69/EC (food) and 2002/70/EC (feed)
  - Also application of bioassays
- Upgraded to Commission Regulations EC (No) 1883/2006 (food) and 152/2009 (feed)
- Recently replaced by Commission Regulations EC (No) 252/2012 (food) and 278/2012 (feed)
- Always based on the work of expert groups
GC/HRMS and/or bioassay

- (DR) CALUX: screening
  - removal negative samples
  - confirmation suspects
- GC/HRMS: confirmation
  - Also GC/MS/MS suitable
CALUX screening assay

[Diagram of CALUX screening assay]

Luciferin + ATP

Aarts et al. 1993
Dose-related response

![Graph showing a dose-response curve with concentration of TCDD (pM) on the x-axis and response (RLUs) on the y-axis. The curve is continuous and the data points are marked with error bars.](image-url)
Application of bioassays

- Most samples are negative and many are even for GC/MS difficult to analyze
- Used for selection of samples
  - Negative or suspected
  - Estimation of level possible but not required
- Also may detect other AhR-agonists
  - False-positive or indication for novel risk?
Screening versus confirmation

- Screening should not miss positive samples
  - Chance less than 5%
- Confirmation should not falsely decide on positive result
  - Chance less than 5%

Decision limit screening  
Decision limit confirmation  
Maximum level  
"False positives"
Effect-based bioassays for screening

Chemical analysis and bioanalysis are complementary !!!
Detection of novel risks: unknown dioxin-like compounds
Eggs during incident with corn (2010)

- Overestimation of levels in eggs, also in the corn
- At least partly caused by non-2,3,7,8-congeners, also in eggs
Cholin Chloride

- Feed additive (up to 1 g/kg)
- Positive test response in DR CALUX (different samples)
- Indicative level around 5 ng BEQ/kg
- GC/HRMS: dioxins and dioxin-like PCBs below LOQ
- Various flame retardants present, including tribromophenols
- But also brominated dioxins, considered equally toxic as chlorinated dioxins
Patterns for source identification

- During incidents very important to discover the source asap
- Patterns can give indication
- Only possible with GC/MS
### Calculation TEQ level: Belgian feed ‘99

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<tr>
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| Total                 | 2883           | 782         |
GC/MS: congener patterns

Fraction of total TEQ (%)

PCB feed Belgium 1999
Different patterns for different sources
Unknown pattern in potato peels (2004)
Identification source

- 2,3,7,8-TCDF
- 2,3,7,8-TCDD
- 1,2,3,7,8-PeCDF
- 2,3,4,7,8-PeCDF
- 1,2,3,7,8-PeCDD
- 1,2,3,4,7,8-HxCDF
- 1,2,3,6,7,8-HxCDF
- 2,3,4,6,7,8-HxCDF
- 1,2,3,7,8,9-HxCDF
- 1,2,3,4,7,8-HxCDD
- 1,2,3,6,7,8-HxCDD
- 1,2,3,7,8,9-HxCDD
- 1,2,3,4,6,7,8-HpCDF
- 1,2,3,4,7,8,9-HpCDF
- 1,2,3,4,6,7,8-HpCDD
- OCDF
- OCDD

fraction of total TEQ (%)
Identification source

- PCB feed Belgium 1999
- Citrus pulp Brazil 1998
- Kaolinic clay Germany 1999
- Cholin chloride 2002 Spain
- Bakery waste 2004 Germany
Exposure to dioxins and dioxin-like PCBs
Exposure to dioxins and PCBs

- Poisoning
- Accidental: environmental, work, food
- Regularly through the food chain
Seveso 1976

- ICMESA chemical plant at Seveso, Italy
  - Production of 2,4,5-trichlorophenol (TCP)
  - On 10 July 1976 emission to an area of 1800 hectares
  - Release of 0.3 – 130 kg dioxins, primarily TCDD
### Seveso

![Map of Seveso](image)

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<th>Zone</th>
<th>Subjects 3–14 Yrs. cases</th>
<th>Chloracne Percent</th>
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<td>A-max(a)</td>
<td>54</td>
<td>26</td>
</tr>
<tr>
<td>B</td>
<td>1,468</td>
<td>8</td>
</tr>
<tr>
<td>R</td>
<td>8,680</td>
<td>63</td>
</tr>
<tr>
<td>R Polo(b)</td>
<td>750</td>
<td>19</td>
</tr>
<tr>
<td>Outside</td>
<td>48,263</td>
<td>51</td>
</tr>
</tbody>
</table>

(a) Includes only the most contaminated part of Zone A.
(b) Sub-zone located near the plant.
Health effects

- Chloracne (193 cases in 1978), children primarily
  - TCDD levels in blood of 2000-56000 pg TEQ/g fat
  - Normally around 20 pg TEQ/g fat, so 100-2800x lower)

- Follow-up studies (still ongoing):
  - Increased incidence soft tissue sarcomas, hemopoietic neoplasms, liver and breast cancer
  - Decreased sperm counts in boys exposed at infancy, reversed for boys exposed at puberty
  - Increased incidence Diabetes mellitus
  - Change of sex ratio (more girls)

- But, exposed group rather small for firm conclusions
Oil disease: YuSho (1967), YuCheng (1979)

- Contamination of rice oil with PCB-oil, used as heat transfer fluid
  - Yusho (Japan) 2000 people exposed
  - Yucheng (Taiwan) 2000 people exposed
    - Used for 9 months
    - Average exposure 1 g PCBs, 4 mg PCDFs
    - TEQ levels around 40 ng TEQ/g fat
- Oil also contaminated with dioxins
- Many people with chloracne
Other incidents in the food chain
1957: chickens discover dioxins

- Millions of dead and diseased chickens in US
- Chicken oedema disease
- After ten years dioxins identified as toxic agent
- Source: fat scrapings from cow hides that were treated with polychlorophenols
- Another chicken incident in 1969 in North Carolina due to wastewater from pesticide plant, with similar symptoms
Dioxins in Dutch milk: waste incineration (1989)

- Sharp decrease of milk levels after improvement incinerators
- Also cases with MWIs and other industries in other countries
- In South Italy problems with mozzarella, due to waste burning
Dioxins in Brazilian citrus pulp (1998)

Reuse of contaminated lime for lowering water content and pH increase
The Belgian dioxin crisis in 1999
Dioxins again discovered by chickens

- Decreased hatching
- No deficiency
## Dioxins & PCBs in feed, chicken and egg

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dioxins* (pg WHO-TEQ/g)</th>
<th>no-PCBs** (pg WHO-TEQ/g)</th>
<th>ind-PCBs*** (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal feed</td>
<td>782</td>
<td>361</td>
<td>32</td>
</tr>
<tr>
<td>Chicken fat</td>
<td>958</td>
<td>453</td>
<td>37</td>
</tr>
<tr>
<td>Egg fat</td>
<td>685</td>
<td>ND</td>
<td>35</td>
</tr>
</tbody>
</table>

*Background levels below 5 pg WHO-TEQ/g fat  
**Planar PCBs reflects the sum of PCBs 126, 169 and 77  
*** sum of PCBs 28, 52, 101, 118, 138, 153, 180, which account for about 30% of the dioxins in the case of a PCB-mixture of Arochlors 1254 and 1260.

Source: 200 l PCB-oil!
Development of the crisis
Testing of samples during the crisis

- Testing started 4 months after the incident
  - Tracking and tracing very difficult
  - All food items suspected, but few contaminated
- Incident became public just before elections
- Effects on consumers?
Study on dioxin and planar PCB levels in eggs

Feed 15x diluted

![Graph showing the levels of total TEQ, mo-PCBs, PCDD/Fs, and no-PCBs over time.](image)
Consequences Belgian crisis

- Highest dioxin/PCB levels in eggs around 8 ng TEQ/g fat, or 50 ng TEQ/egg
- Consumption of 1 egg/day for 1 week:
  - 350 ng TEQ/week or 5000 pg TEQ/kg bw/week
  - (TWI 14 pg TEQ/kg/wk): 350x lower
- Effect on body burden?
  - Existing body burden: 300 ng TEQ (15 kg body fat; 20 pg TEQ/g fat)
  - Possibly 2-3 fold increase of body burden
  - Still much lower than in Seveso
  - Difficult to predict subtle adverse effects
Other consequences Belgian dioxin crisis

- Major impact on Belgian economy
  - 500-600 m€ financial damage (EU compensation)
- Whitebook
  - Establishment of EFSA (European Food Safety Authority)
  - General food law (Regulation (EC) No 178/2002)
  - GMP for feed
  - Limits for dioxins and later dl-PCBs in food and feed
- Dioxins became politically very sensitive
Limits for food and feed
Limits for food and feed

- Current exposure population around exposure limit
- Food levels should be further reduced
  - Limits should not result in high non-compliance rates: “strict but feasible”
- Eventual goal is reduction of exposure below TWI
Establishment of EU-limits

- Inventory of existing levels
  - First dioxins
  - Later dioxin-like PCBs
  - Limit around 95\textsuperscript{th} percentile; so 5\% above limit
- Result: different MLs for different species
- Also action limits (2/3 of limit)
  - Follow-up recommended
Food limits (since July 2002)

Many different limits
- Based on TEFs 1998
- Limits for pork (1), poultry (2), beef, milk and eggs (3), expressed in pg TEQ/g fat
- Limit for fish: 4 pg TEQ/g fish

First only dioxins; since 4-11-2006 also dl-PCBs
- ML for sum dioxins and dl-PCBs, and for dioxins but not for dl-PCBs
- AL for dioxins and for dl-PCBs, not for the sum
Action limits and maximum limits

- **ML Total TEQ**: 1.5 pg TEQ/g
- **ML dioxins**: 1.0 pg TEQ/g
- **AL dioxins**: 0.6 pg TEQ/g
- **AL dl-PCBs**: 0.5 pg TEQ/g
New limits since January 2012)

- Often lower
- Application of “new” TEFs 2005
## Maximum and action levels old/new

<table>
<thead>
<tr>
<th>Product</th>
<th>AL Dioxins</th>
<th>AL PCBs</th>
<th>ML dioxins</th>
<th>ML Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pork</td>
<td>0.6</td>
<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>0.5</td>
<td>1.0</td>
<td>1.25</td>
</tr>
<tr>
<td>Poultry</td>
<td>1.5</td>
<td>1.5</td>
<td>2.0</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>0.75</td>
<td>1.75</td>
<td>3.0</td>
</tr>
<tr>
<td>Beef</td>
<td>1.5</td>
<td>1.0</td>
<td>3.0</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>1.75</td>
<td>1.75</td>
<td>2.5</td>
<td>4.0</td>
</tr>
<tr>
<td>Eggs</td>
<td>2.0</td>
<td>2.0</td>
<td>3.0</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>1.75</td>
<td>1.75</td>
<td>2.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Milk</td>
<td>2.0</td>
<td>2.0</td>
<td>3.0</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>1.75</td>
<td>2.0</td>
<td>2.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Fish</td>
<td>3.0</td>
<td>3.0</td>
<td>4.0</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td></td>
<td></td>
<td>6.5</td>
</tr>
</tbody>
</table>
Intention to further lower the limits

- MLs by themselves do not guarantee that exposure is below TWI
- Eg. Consumption of 300 gram fish recommended per week
  - ML sum-TEQ is 6.5
  - 300 g at ML would mean intake of 1950 pg TEQ
  - Or about 30 pg TEQ/kg bw i.e. 2x TWI for fish only
  - In practice levels in most fish well below MLs
- Initial plan was setting of target levels but abandoned
Compliance with MLs

- Most products well below limits (by definition), except:
  - Part of the wild eel
  - Part of free-range eggs
    - Due to soil consumption by hens
    - Still various farm-related incidents
- during food/feed incidents
Questions?