CALUX 2010

Dr. Peter A. Behnisch
Director of BDS b.v.
Working Experiences at Universities

• 1992-1997: PhD at University Tuebingen, Germany

• 1998: Postdoc at Prof. K. Jones, Lancaster University, England
Working Experiences at Kaneka

• 1998-2002: Kaneka Corp., Kobe, Kapan –

Bioassay Lab:

CALUX
E-screen
EROD
Working Experiences at SGS

- 2002-2004: Head of Laboratory of SGS Control. Co in Hamburg and Wismar

- Establishment SGS Dioxin Lab in Ludington, USA
2004-2005: Head of Laboratory at Eurofins-GfA, Muenster and Hamburg, Germany
Welcome to the Amsterdam Sciencepark
BioDetection Systems B.V. (“BDS”) is a Dutch company providing biological detection systems, such as the innovative CALUX® bioassays for the determination of ultra low levels of a variety of highly potent materials.

Mission
To provide innovative bioassays and implement their use to the highest international standards.

Activities:
• ISO 17025 accredited Laboratory – Contract analysis
• Licensing
• Training
• Research and Development
• Consultancy

Ankara, Turkey, 30 March 2010
Effect-based analysis offers more than the top of the ice mountain by chemical analysis...

- **Substances:**
  - selected priority pollutants

- **Effects:**
  - General toxicity: effects of total mixture of pollutants
  - Specific toxicity: effects of substances with a similar mechanism of toxic action
  - Unknown cause of effect (TIE needed)

More reliable risk assessment by use of toxic screening prior to relevant chemical analyses
CALUX- biosensors, main marketed products of BDS

Add substrate (luciferine)

Proteins
Enzymes
Luciferase

Chemical receptor

Transport protein

Ligand binding

Chemical Responsive Element (CRE)

Nucleus
Cytosol

Transcription

Hsp
Development of array of steroid reporter gene assays (STERoLUX)

- Human U2OS-based
- Stable human steroid receptor expression
- Minimal reporter constructs
- High selectivity and specificity
# CALUX screening battery

<table>
<thead>
<tr>
<th>Name</th>
<th>Examples applications</th>
<th>Ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR CALUX</td>
<td>Clinical, food, environment, reproduction, cancer</td>
<td>Dioxins and dioxin-like chemicals</td>
</tr>
<tr>
<td>PAH CALUX</td>
<td>Clinical, food, environment, reproduction, cancer</td>
<td>Carcinogenic PAHs</td>
</tr>
<tr>
<td>ER CALUX</td>
<td>Clinical, food, pharma, environment, reproduction, cancer</td>
<td>Estrogens, EDCs</td>
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<td>ERalpha CALUX</td>
<td>Clinical, food, pharma, environment, reproduction, cancer</td>
<td>Estrogens, EDCs</td>
</tr>
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<td>ERbeta CALUX</td>
<td>Clinical, food, pharma, environment, reproduction, cancer</td>
<td>(Phyto)Estrogens, EDCs</td>
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<td>AR CALUX</td>
<td>Clinical, food, pharma, environment, reproduction, cancer</td>
<td>Androgens, EDCs</td>
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<tr>
<td>PR CALUX</td>
<td>Clinical, food, pharma, environment, reproduction, cancer</td>
<td>Progestins, EDCs</td>
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<td>GR CALUX</td>
<td>Clinical, food, pharma, environment, doping, inflammation</td>
<td>Glucocorticoids, EDCs</td>
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<td>TR CALUX</td>
<td>Clinical, food, pharma, environment, energy metabolism</td>
<td>Thyroid hormones, EDCs</td>
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<tr>
<td>RAR CALUX</td>
<td>Clinical, food, pharma, reproduction, cancer, teratogenicity, cosmetics</td>
<td>Retinoids</td>
</tr>
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<td>PPAR CALUX</td>
<td>Clinical, food, pharma, environment, cancer, metabolic syndrome</td>
<td>Wide range</td>
</tr>
<tr>
<td>kappaB CALUX</td>
<td>Clinical, food, pharma, environment, inflammation, stress</td>
<td>Pro-inflammatory cytokines</td>
</tr>
<tr>
<td>P21 CALUX</td>
<td>Clinical, food, pharma, environment, cell/DNA damage</td>
<td>Genotoxic agents</td>
</tr>
<tr>
<td>Cytox CALUX</td>
<td>Environment, food, pharma, cytotoxicity, specificity control</td>
<td>Cytotoxic agents</td>
</tr>
<tr>
<td>Nrf2 CALUX</td>
<td>Clinical, food, pharma, environment, cancer, cell protection</td>
<td>Electrophiles, ox stress</td>
</tr>
<tr>
<td>P53 CALUX</td>
<td>Clinical, food, pharma, environment, cell/DNA damage</td>
<td>Cytotoxic agents</td>
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<td>AP1 CALUX</td>
<td>Clinical, food, pharma, environment, reproduction, cancer</td>
<td>Carcinogens, UV</td>
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<tr>
<td>Etc..</td>
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</table>
Applications CALUX® battery

- Acute
  - KappaB nrf2 cytox p53 p21

- Immuno
  - ER AR PR GR RAR DR KappaB nrf2 cytox PPAR

- Muta/carcinogen
  - ER AR PR TR GR RAR DR KappaB nrf2 cytox PPAR p53 p21

- Endocrine
  - ER AR PR TR GR RAR DR KappaB cytox PPAR

- Repro
  - ER AR PR TR GR RAR DR KappaB nrf2 cytox PPAR p53 p21

- Development
  - ER AR TR GR RAR DR KappaB cytox PPAR
Screening technologies applied in EC monitoring and R&D projects

- **Food and Feed (safety/functional foods)**
  - EU Project DIFFERENCE – dioxin/PCB screening in food/feed
  - EU project Plantlibra- beneficial food ingredients
  - Dutch Food and Nutrition project-tests for beneficial food ingredients

- **Water**
  - Technological collaboration project Economic affairs – genomics-based biodetection
  - EU Project TECHNEAU – water safety
  - EU Project ACE – what to do with complex mixtures of pollutants?
  - Dutch project Genes for Water- water safety

- **Environment**
  - Dutch Projects Ecogenomics – healthy soil, DNA barcoding
  - EU Project FACE IT – early warning oil spill biotests
  - EU Project HORIZONTAL – dioxin/PCB screening in soil, sludge/biowaste
  - Belgium DISCRISET Project – rapid testing for hazardous waste
  - Japanese MILLENIUM Project for safe waste recycling technologies
  - Swiss Project: Global warming – how to make car exhaust gas safer?

- **Chemicals and biologicals (safety/discovery)**
  - EU Project FIRE: brominated flame retardants
  - EU Project REPROTECT – non animal testing for REACH
  - EU project METAEXPLORE- metagenomics
  - EU project CHEMSCREEN- non animal testing for REACH
  - Netherlands Toxicogenomics Centre- genomics and non animal testing for chemical safety

- **Human health (clinical/epidemiology/doping)**
  - Wada project- antidoping
  - EU Project NEW GENERIS – Baby/mother health biomarkers

- **Pharmaceuticals (safety/discovery)**
  - Dutch Projects EcoLinc – metagenomics approaches
  - Top Institute Pharma project – tests for adverse drug reactions/metabolism
  - Netherlands Toxicogenomics Centre- genomics and non animal testing for drug safety
<table>
<thead>
<tr>
<th>Name</th>
<th>Topic</th>
<th>CALUX</th>
<th>Period/homepage</th>
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<tbody>
<tr>
<td>FP 6 Facelt</td>
<td>Oil spill - early warning and toxicity</td>
<td>PAHs</td>
<td>2005-2009 <a href="http://www.unil.ch/face-it">www.unil.ch/face-it</a></td>
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<td>Hazardous waste</td>
<td>DR</td>
<td>2008-2011 <a href="http://www.vito.be">www.vito.be</a></td>
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<td>FP 6 ReProtect</td>
<td>REACH</td>
<td>ER, AR</td>
<td>2006-2009 <a href="http://www.reproprotect.eu">www.reproprotect.eu</a></td>
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<td>FP 6 Techneau</td>
<td>EDC water</td>
<td>CALUX panel</td>
<td>2008-2009 <a href="http://www.techneau.org">www.techneau.org</a></td>
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<td>FP 6 NewGeneris</td>
<td>Mother-newborn baby health biomarker</td>
<td>DR, ER, AR</td>
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<td>Soil quality</td>
<td>CALUX panel</td>
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<td>Food and Nutrition Delta</td>
<td>Bioassays for health claims functional foods</td>
<td>Various novel</td>
<td>2008-2010 <a href="http://www.foodnutritiondelta.nl">www.foodnutritiondelta.nl</a></td>
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<td>Netherlands Toxicogenomics Centre</td>
<td>REACH-alternative to animal tests</td>
<td>Various novel</td>
<td>2008-2012 <a href="http://www.toxicogenomics.nl">www.toxicogenomics.nl</a></td>
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<tr>
<td>FP7 Metaexplore</td>
<td>New biofunctionals</td>
<td>Various</td>
<td>2009-2013</td>
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</table>
North America
- USA: 1

South America: 2

Europe
- Belgium: 3 Labs
- Denmark: 1
- Sweden: 1
- Germany: 2 Labs
- Czech Republic: 1
- Slovakia: 1
- Switzerland: 2
- Italy: 2
- Cyprus: 1
- France: 2
- Netherlands: 6 Labs
- Belgium: 3 Labs

Asia
- Japan: 3 Labs
- China: 2
- Vietnam/Korea: 2
- Taiwan: 4 Labs
- New Zealand: 1
- Australia: 2
- Thailand: 1
- Turkey: Edip Sincer

Consultants
- Service laboratories
- Laboratories

International acceptance……
RESULTS OF THE FIRST INTERNATIONAL INTERLABORATORY DR CALUX® by BDS COMPARISON STUDY FOR FOOD AND FEED (BICS 2005).

Besselink HT, Felzel E, Jonas A and Brouwer A

BioDetection Systems BV (BDS), Kruislaan 406, 1098 SM Amsterdam, The Netherlands

Introduction

Food and feed safety is a high priority issue for the food and feed sector as it directly impacts on human and animal health. Stringent EU limit values are in force for dioxins in food- and feedingstuffs\textsuperscript{1,2} for animal and public health protection. The use of the DR CALUX\textsuperscript{®} by BDS bioassay for monitoring dioxins in food and feed allows the (pre)-selection of samples suspected of being contaminated above limit values with dioxins. To permit bioassays to be used for screening of food- and feedingstuffs, the EU has laid down general requirements for the determination of dioxins and dioxin-like PCBs in food- and feedingstuffs and specific requirements for cell-based bioassays\textsuperscript{3,4}. To ensure the reliability and performance of the DR CALUX\textsuperscript{®} by BDS bioassay for monitoring food and feedingstuffs, an interlaboratory comparison study (ringtest) is mandatory.

In the present paper, the results of the first international DR CALUX\textsuperscript{®} interlaboratory comparison study (BICS 2005) organized by BioDetection Systems BV (BDS) are described. A total of 21 laboratories world wide using the DR CALUX\textsuperscript{®} bioassay in house participated in the BICS-2005 study.

A total of 21 laboratories were invited and participated in the BICS-2005 study:

- AgriQuality Ltd., Lower Hutt, New Zealand
- Bureau of Food and Drug, Nangang Taipei, Taiwan
- C.A.R.T.- University of Liege, Liege, Belgium
- CCL B.V., Veghel, The Netherlands
- CEFAS, Burnham-on-Crouch, United Kingdom
- DWR, Amsterdam, The Netherlands
- EMPA, Dübendorf, Switzerland
- Environmental Analysis Laboratory of EPA, Chung Li City, Taiwan
- Instituto Superiore di Sanita, Rome, Italy
- Kaneka Techno Research Co., Ltd., Takasago-city, Japan
- Keuringsdienst van Waren, Zutphen, The Netherlands
- LABTRASA, Murcia, Spain
- Masterlab BV, Boxmeer, The Netherlands
- NIES, Tsukuba-city, Japan
- Public Analyst's Laboratory, Galway, Republic of Ireland
- RIKILT, Wageningen, The Netherlands
- SGIT-INIA, Madrid, Spain
- State Veterinary and Food Institute, Kosice, Slovakia
- Veterinary Research Institute, Brno, Czech Republic
- VITO, Mol, Belgium
- BioDetection Systems, Amsterdam, The Netherlands
Outline of Presentation

- Types and purposes of dioxin surveillances
- Crisis related monitoring
- Source directed Survey
- EU perspective on managing dioxin contamination
- Background Survey/Trend analysis
- Initial results from Chilean survey
- Total Dietary/targeted Survey (TDS/non-TDS)
- Human/environmental monitoring
- Future perspectives EU
### Overview types and purposes of dioxin surveillances

<table>
<thead>
<tr>
<th>Type</th>
<th>Purpose</th>
<th>Sector</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crisis related monitoring</td>
<td>Management of crisis with purpose to resolve swiftly</td>
<td>Import/Export of Food, Feed, Raw materials</td>
<td>Incidental</td>
</tr>
<tr>
<td>Source directed Survey</td>
<td>Source identification</td>
<td>Environment, Feed, Food</td>
<td>Incidental/longer term</td>
</tr>
<tr>
<td>Background Survey</td>
<td>Source identification</td>
<td>Environment, Feed, Food</td>
<td>Incidental/longer term</td>
</tr>
<tr>
<td>Trend analysis</td>
<td>Source identification</td>
<td>Environment, Feed, Food</td>
<td>Incidental/longer term</td>
</tr>
<tr>
<td>Total Dietary Survey (TDS)</td>
<td>Background levels reductions</td>
<td>Food, Feed, Raw materials</td>
<td>Long term</td>
</tr>
<tr>
<td>Targeted Survey</td>
<td>Dietary intake estimation</td>
<td>Food, Nutrition, Public Health</td>
<td>Long term</td>
</tr>
<tr>
<td>Human monitoring</td>
<td>Human risk assessment</td>
<td>Public Health</td>
<td>Long term</td>
</tr>
<tr>
<td>Environmental Monitoring</td>
<td>Environmental risk evaluation</td>
<td>Environmental Health</td>
<td>Long term</td>
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<tr>
<th>Country</th>
<th>Product/Ingredient</th>
<th>Year</th>
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<tbody>
<tr>
<td>Brazilian</td>
<td>Citrus pulp</td>
<td>1998</td>
</tr>
<tr>
<td>Belgian</td>
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<td>Kaolinic clay</td>
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<td>Cholin chloride</td>
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<td>Guar Gum/PCP</td>
<td>2007</td>
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<tr>
<td>Italy</td>
<td>Mozzarella/waste disposal</td>
<td>2008</td>
</tr>
</tbody>
</table>
Characteristics of Dioxin crisis situations (in the past)

• Dioxins are not acute toxicants: therefore usually there is an extended time-lag between initial contamination and discovery

• Dioxins are toxic at very low levels: requires expensive, sophisticated analysis which is performed at relatively low frequency

• Sudden release in the media of dioxin contamination causes panic in general public (health concern), government (political consequences) and industry (export ban/ recall)

• Extend of contamination is unclear for extended time, due to lack of data/low analysis capacity/extended turn around times

• Trans-industrial and trans-national spreading of the problem far beyond original source, many possible sources, whole chain contamination
Characteristics of Dioxin crisis situations (present and future)

• Fast, low cost analysis methods available (e.g., CALUX) enabling high capacity screening and surveillance of food, feed and environment thus:

  • reducing time-lag between initial contamination and discovery
  
  • enabling medium-high frequency analysis at relatively low cost and high capacity

  • swift analysis of extend of contamination and source identification

  • much faster and more robust information available on short notice to manage and reduce public concern, enhance lift of-, or even prevent export ban/recall

  • prevent, or reduce spreading of the problem to trans-industrial and trans-national level
Performance of DR CALUX®:
good comparison to HR-GCMS
fulfills EU requirements, ISO 17025 accredited
<table>
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<th>Country</th>
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</table>
Belgian dioxin chicken crisis 1999

Cause: illegal mixing of industrial oil (PCBs) with recycled fats for feed production
CALUX bioassay results:

- Negative results: 2107 (87%)
- Suspected samples: 274 (11%)
- Samples that could not be tested: 30 (1%)
- Suspected samples investigated by HRGC/HRMS (n=136):
  - 53% positive samples confirmed for only dioxin values
  - If dioxin-like PCBs have been included nearly all samples
    have been confirmed!
- Control of false negative samples (n=141):
  - Only 1 positive = < 1%

Conclusion: Ideal situation for a rapid screening method!
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<tr>
<td>Italy</td>
<td>Mozzarella/waste disposal</td>
<td>2008</td>
</tr>
</tbody>
</table>
Dutch Dioxin crisis 2004:
Clay effecting potato peelings used in animal feed.

No of samples analyzed during crisis (4 months) several thousands

total DR CALUX TEQ distribution for milk samples, 2004 Dutch clay crisis

60% below 1.5 pg TEQ/g

5.8% above 3 pg TEQ/g

1.3% above 6 pg TEQ/g

reported TEQ (sorted from lowest to highest)
THE MOZZARELLA CHEESE INCIDENT

The monitoring plan design (Scotticini)

Phase I-II samples:
milk from a single farm or pooled milk from 2 - 4 farms.
THE MOZZARELLA CHEESE INCIDENT
PCDD/Fs levels in MILK (Phase I-II Scotticini)

pg WHO-TEQ/g fat
- < 1.0
- 1.0 - 2.0
- 2.0 - 3.0
- > 3.0
The Eggs contamination case (Scotticini, 2004)
Results on the samples taken at the farm

1. **Eggs** 33 pg WHO-TEQ/g fat, **laying hens meat** 45 pg WHO-TEQ/g fat, thus confirming the contamination found in the previously analysed egg sample.

2. **Cheese** (from bovine milk) 0.50 pg WHO-TEQ/g fat, (environmental pollution excluded).

3. **Poultry feed** 0.05 pg WHO-TEQ/g (contamination due to feed products excluded).

4. **Wood-shaving litter** 51 pg WHO-TEQ/g, **wood shavings** 40 pg WHO-TEQ/g, thus obtaining the identification of the contamination source.
**History of Italian „Mozarella Crisis“(2008)**

- The NRL of Italy IZSAM started their Residence Surveillance Plan in 1999 with about 50 samples for PCDD/Fs for food.

<table>
<thead>
<tr>
<th></th>
<th>a) 2000-03</th>
<th>b) 2004-06</th>
<th>c) 2008</th>
<th>c) Plan 2009</th>
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<td>PCDD/F-TEQ</td>
<td>Media</td>
<td>all IZS</td>
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<td><strong>Meat</strong></td>
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<tr>
<td>Chicken</td>
<td>74</td>
<td>0.1-1.9</td>
<td>134</td>
<td>0.1-1.0</td>
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<td></td>
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<tr>
<td>Bovine</td>
<td>68</td>
<td>0.1-3.7</td>
<td>82</td>
<td>0.1-1.7</td>
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<td>Pork</td>
<td>108</td>
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<td>146</td>
<td>0.1-0.5</td>
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<td>123</td>
<td>0.1 - 2.9</td>
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<td>0.1-0.8</td>
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<td>Sheep/Goat</td>
<td>18</td>
<td>0.1 - 6.2</td>
<td>49</td>
<td>0.1 - 30</td>
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<td>0</td>
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<td>50</td>
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<td><strong>Fish</strong></td>
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<td>0.1-0.8</td>
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<td>16</td>
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<td>17</td>
<td>0.1-0.8</td>
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<tr>
<td>Salmon</td>
<td>0</td>
<td>nd</td>
<td>6</td>
<td>0.1-0.3</td>
</tr>
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<td>Others</td>
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</tr>
</tbody>
</table>

**Samples/year ca.:**
- 180
- 310
- 1200
- 3000
Conclusions

a) Wood shavings were obtained from wood imported from Cameroun and treated with pentachlorophenol as preservative.

b) 1,2,3,4,6,7,8-HpCDD and OCDD are by-products in the synthesis of pentachlorophenol and also formed by photolysis of pentachlorophenol.

c) The congener profile of wood shavings (and litter) overlapped with those of eggs and meat.

d) It was concluded that the wood treatment with pentachlorophenol caused the contamination of laying hens.
Outline of Presentation

• Types and purposes of dioxin surveillances
• Crisis related monitoring
• **Source directed Survey**
• EU perspective on managing dioxin contamination
• Background Survey/Trend analysis
• Initial results from Chilean survey
• Total Dietary/targeted Survey (TDS/non-TDS)
• Human/environmental monitoring
• Future perspectives EU
Main sources of dioxins:

- (Hospital) Waste incinerators
- Iron ore sintering
- Electric arc furnaces
- Non-ferrous metal industry (AL, CU)
- Cement kilns
- Domestic solid fuel combustion
- Backyard burning
- Natural sources (clay, mines, volcanoes, forest fires)
- Burning animal carcasses
- Wastes / byproducts of chloro-alkali-industry
How to identify sources of dioxin contamination

- HR-GCMS is the best tool for source identification (pattern fingerprinting)

- Bioassays (e.g. CALUX) are best tool for measuring location, extent and frequency of contamination (e.g. by combining bioassay results with GPS-area mapping)
Outline of Presentation

• Types and purposes of dioxin surveillances
• Crisis related monitoring
• Source directed Survey
• **EU perspective on managing dioxin contamination**
• Background Survey/Trend analysis
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• Human/environmental monitoring
• Future perspectives EU
A 10-year plan is established in the EU to reduce dioxins and dl-PCBs in food

Food of animal origin is predominant source of human exposure

Since food contamination is directly related to feed contamination an integrated approach is followed to reduce dioxin/PCB incidents all along the food chain

Estimate background levels in order to identify environmental sources with the aim to limit the release of dioxins/PCBs into the environment
There are three pillars in food and feed legislative measures

• The establishment of maximum levels at a strict but feasible level in food and feed

• The establishment of action levels acting as a tool for “early warning” of higher than desirable levels of dioxins/PCBs in food and feed

• The establishment of target levels, over time, to bring exposure of a large part of the EU population within the TDI and TWI limits
### Tolerance and action limits since 2006

<table>
<thead>
<tr>
<th>Product</th>
<th>Tolerance limit</th>
<th>Tolerance limit</th>
<th>Action limit</th>
<th>Action limit</th>
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COMMISSION RECOMMENDATION

of 16 November 2006

on the monitoring of background levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs

(notified under document number C(2006) 5425)

(Text with EEA relevance)

(2006/794/EC)
Overview of the recommended minimum number of food samples to analyse yearly (background survey).

<table>
<thead>
<tr>
<th>Product, including also derived products</th>
<th>Aquaculture (*)</th>
<th>Wild caught fish (**)</th>
<th>Fruit (***+)</th>
<th>Milk (****)</th>
<th>Eggs (*****+)</th>
<th>Other (******+)</th>
<th>Total</th>
</tr>
</thead>
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<td>267</td>
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<td><strong>485</strong></td>
<td><strong>500</strong></td>
<td><strong>250</strong></td>
<td><strong>250</strong></td>
<td><strong>267</strong></td>
<td><strong>2000</strong></td>
</tr>
</tbody>
</table>
Guideline: 2006/794/EC recommendations for sampling

- Distribution of samples is based on production in each country.
- Particular attention is paid to foodstuffs expected to have a large variation in background levels of dioxins, furans and dioxin-like PCBs.
- This is particularly the case for fish.
- Aquaculture: The samples for aquaculture should be divided over the fish species proportionate to the production.
- As guidance, the species specific data on production of fish and fishery products ‘Facts and Figures on the CFP — basic data on the Common Fisheries Policy’ (1), European Communities, 2006 and the map ‘Aquaculture in the European Union’ (2). can be used.
- Special attention should be paid to oysters, mussels and eel.
- **Meat:** In addition to meat and meat products originating from beef cattle, pigs, poultry and sheep, significant number of samples should be taken from horsemeat, reindeer meat, goat meat, rabbit meat, venison and game.
EU recommendation Feed Monitoring

Feed Monitoring

COMMISSION RECOMMENDATION
of 11 October 2004
on the monitoring of background levels of dioxins and dioxin-like PCBs in feedingstuffs
(notified under document number C(2004) 3461)
(Text with EEA relevance)
(2004/704/EC)
ANNEX I – EC/704/2004 Overview of the recommended minimum number of feed samples to analyse yearly.

<table>
<thead>
<tr>
<th>Country(*)</th>
<th>Total number samples recommended for each country</th>
<th>Plant origin</th>
<th>Feed materials, additives, premixtures</th>
<th>Animal origin</th>
<th>Total</th>
<th>Compound feedingstuffs</th>
<th>Terrestrial animals</th>
<th>Fish</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cereals, grains, their products and by-products</td>
<td>Oil seeds of fruits, their products and by-products</td>
<td>Trace elements, vitamins, and feed additives</td>
<td></td>
<td>Animal fat, milk and milk products, fish oil</td>
<td>Cattle</td>
<td>Pig</td>
<td>Poultry</td>
</tr>
<tr>
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</table>

(*) The Czech Republic, Estonia, Cyprus, Latvia, Lithuania, Hungary, Malta, Poland, Slovenia and Slovakia joined the European Community on 1 May 2004. It is appropriate that the new Member States participate in the monitoring programme as soon as possible. It is however acknowledged that it is appropriate to foresee a transitional arrangement for these new Member States and therefore no detailed minimum frequency for the random monitoring of the presence of dioxins, furans and dioxin-like PCBs in feedingstuffs is recommended for these countries.
In the „Other“ category particular attention should be paid to:

- food supplements (particular those ones based on marine oil),
- food for infants and young children,
- food products originating from regions where due to e.g. climatic conditions resulting in floods, changes have happened in the production conditions which could possibly affect the dioxin and dioxin-like PCB concentration of the food products in the region.
Outline of Presentation

- Types and purposes of dioxin surveillances
- Crisis related monitoring
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- Human/environmental monitoring
- Future perspectives EU
Where is the application zone of CALUX with respect to EU limit values for feed and food

Example: National Monitoring Plans for MILK:

regulated level 3 pg TEQ\textsubscript{pcdd/f} or 6pg TEQ\textsubscript{total}/g fat

EC Dioxin/dl-PCB TEQ = ca. 2-5% samples = confirmative HRGC/MS

EC Dioxin/dl-PCB TEQ – 25% = 4.8 = 95-98% samples = DR CALUX

EC Total TEQ – 30% = 4.2

EC Dioxin TEQ in milk = 5-times background level

CALUX level PCB-, dioxin- and total-TEQ accepted by EC/1883/2006

SCAN EC Report 2000: Normal background milk levels
Background survey: Distribution of DR CALUX® TEQ in food samples (n=490) EU countries
Background survey: Distribution of DR CALUX® TEQ levels in feed samples (n=100) EU countries

<p>| | |</p>
<table>
<thead>
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<tr>
<td>Median</td>
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<tr>
<td>% below 0.5</td>
<td>58</td>
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<td>% below 1.0</td>
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<td>% above 1.0</td>
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<td>% above 2.0</td>
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> 4.0; n=2
Background Survey: National Monitoring Program from State Veterinary and Food Service of Slovak Republic

Ratio: Total DR CALUX TEQ vs. accepted European Total-TEQ

Bovine Meat

Pork Meat
Background Survey: National Monitoring Program from State Veterinary and Food Service of Slovak Republic

Ratio: Total DR CALUX TEQ vs. accepted European Total-TEQ

Egg

Milk

Location

Location
Outline of Presentation

• Types and purposes of dioxin surveillances
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• Future perspectives EU
Total DR CALUX® distribution for FEED samples, 2009 Asprocer Program, Chile

- Maximum EU limit PCDD/F-PCB = 1.5 ng TEQ/kg product
- Maximum EU limit PCDD/F = 0.75 ng TEQ/kg product

Santiago, Chile, 22 March 2010
Total DR CALUX® distribution for MINERAL samples, 2009 Asprocer Program, Chile

- Max EU limit PCDD/F-PCB = 1.5 ng TEQ/kg product
- Max EU limit PCDD/F = 0.75 ng TEQ/kg product

38 ng TEQ/kg product

5.4% above max EU PCDD/F-PCB limit
7.6% above max EU PCDD/F limit
75% max EU PCDD/F limit

Santiago, Chile, 22 March 2010
Total DR CALUX® distribution for FISH OIL samples, 2009 Asprocer Program, Chile

- 0% above max EU PCDD/F-PCB limit
- max EU limit PCDD/F-PCB = 24 ng TEQ/kg product

- 0% above max EU PCDD/F limit
- max EU limit PCDD/F = 6 ng TEQ/kg product

- 75% max EU PCDD/F limit

Santiago, Chile, 22 March 2010
Total DR CALUX® distribution for ANIMAL OIL samples, 2009 Asprocer Program, Chile

Santiago, Chile, 22 March 2010
Total DR CALUX® distribution for VEGATABLE OIL samples, 2009 Asprocer Program, Chile

- 0% above max EU PCDD/F-PCB limit
- max EU limit PCDD/F-PCB = 1.5 ng TEQ/kg product
- 18% above max EU PCDD/F limit
- max EU limit PCDD/F = 0.75 ng TEQ/kg product
- 75% max EU PCDD/F limit

Santiago, Chile, 22 March 2010
Outline of Presentation

- Types and purposes of dioxin surveillances
- Crisis related monitoring
- Source directed Survey
- EU perspective on managing dioxin contamination
- Background Survey/Trend analysis
- Initial results from Chilean surveillance
- Total Dietary/targeted Survey (TDS/non-TDS)
- Human/environmental monitoring
- Future perspectives EU
Human exposure to dioxins and dl-PCBs

• The most important route of human exposure to dioxins and dl-PCBs is food consumption

• Food consumption contributes > 90% of total exposure

• Fish and food of animal origin account for > 80% of the overall exposure
Tolerable human intake of dioxins

- **Tolerable daily intake (TDI) for dioxins and dl-PCBs in EU:**
  - 2 pgTEQ/kg body weight per day

- **Tolerable weekly intake (TWI) for dioxins and dl-PCBs in EU:**
  - 14 pgTEQ/kg body weight per week

- **Average dietary intake dioxins and dl-PCBs in EU:**
  - 1,2 – 3 pgTEQ/kg body weight per day

- A considerable part of EU population does exceed TDI, or TWI
Effect of single high dose intake on body burden is relatively small.

Short term variation in intake is relatively unimportant.

Human intake estimates based on TDS foods intake

PCDD/PCDF Exposure Estimates from 2001-2004 TDS Foods

From: www.cfsan.fda.gov/~lrd/dioxecn.html
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Target limits, action limits and residu limits

Residu limit Total TEQ

Residu limit dioxins

Action limit dioxins

Action limit dl-PCBs

1.5 pg TEQ/g

1.0 pg TEQ/g

0.6 pg TEQ/g

0.5 pg TEQ/g

0.1 pg TEQ/g

Action limits ideally the target of screening
Expected future developments in EU legislation

• Implementation of re-evaluated TEF values for existing 27 dioxins/dl-PCBs by WHO in 2011?

• Regulation of food and feed maximum limits on basis of total TEQ only in 2010?

• Inclusion of new, emerging dioxin-like compounds in TEF-concept and expanding analysis to include eg, brominated- and mixed chloro/bromo dioxins 2011?