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

BDS

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

Working experiences at Eurofins

2004-2005: Head of Laboratory at Eurofins-GfA, Muenster and Hamburg, Germany

Welcome to the Amsterdam Sciencepark

BDS company profile

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.

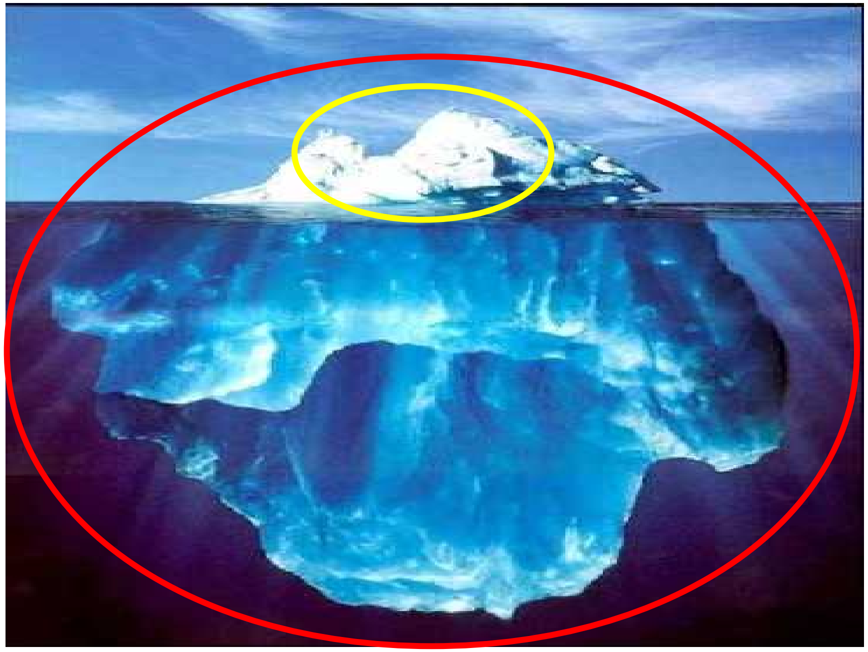
Mission

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**

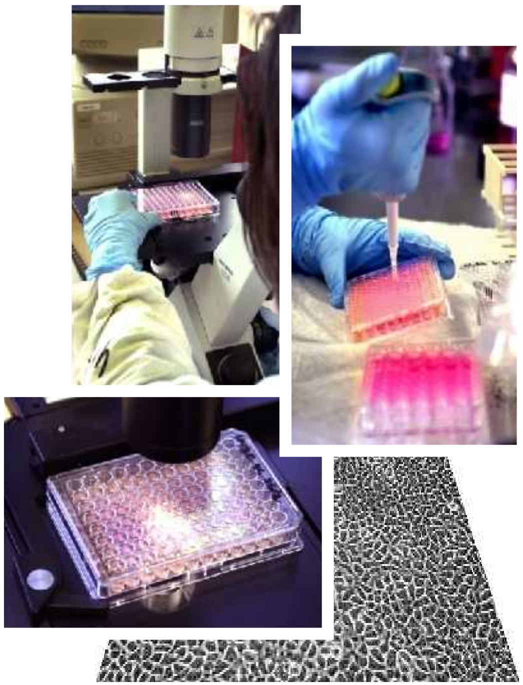
Effect-based analysis offers more than the top of the ice mountain by chemical analysis…

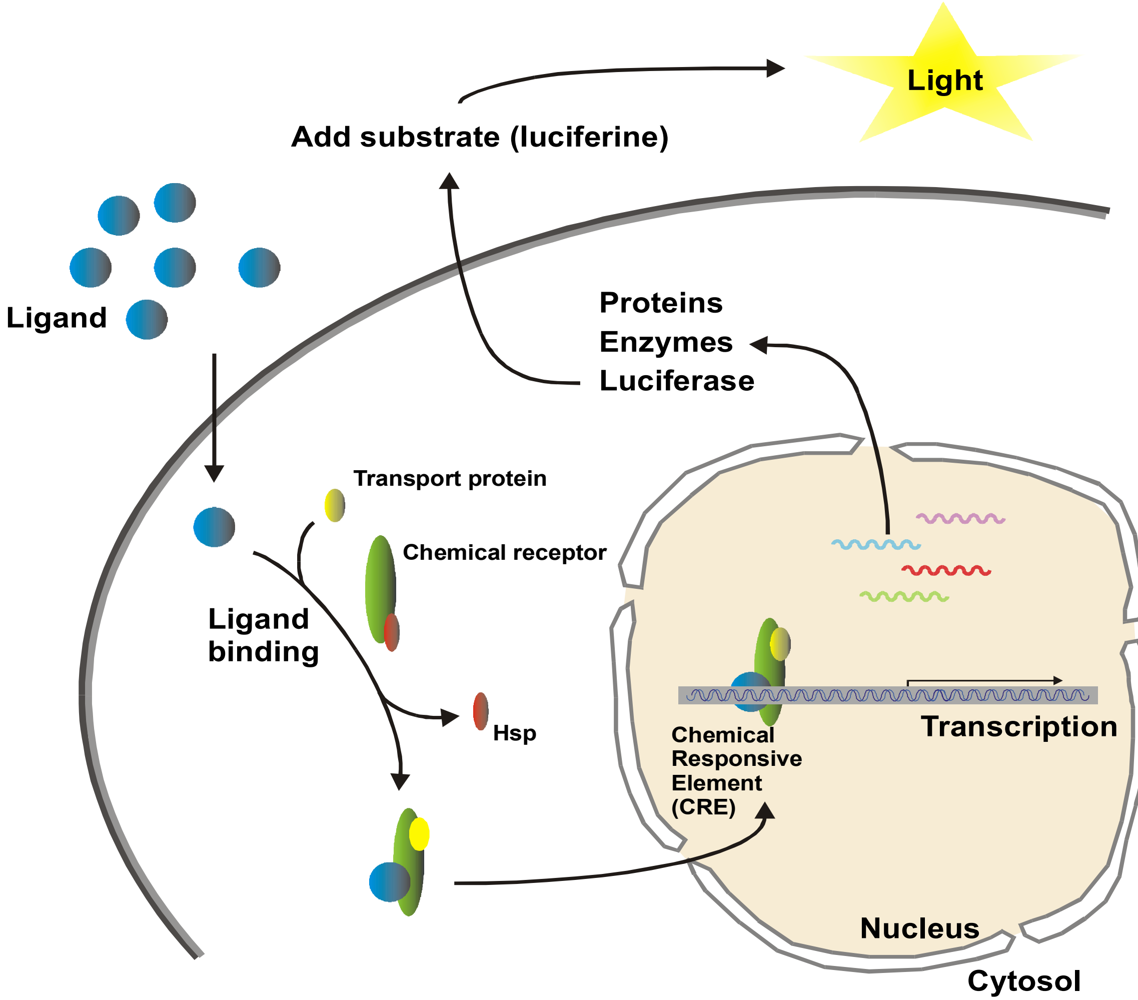


- Substances:
  - selected priority pollutants
- Effects:
  - General toxicity: effects of total mixture of pollutants
  - Specific toxicity: effects of substances with a similar mechanism of toxic action
  - Unknown cause of effect (TIE needed)

More reliable risk assessment by use of toxic screening prior to relevant chemical analyses

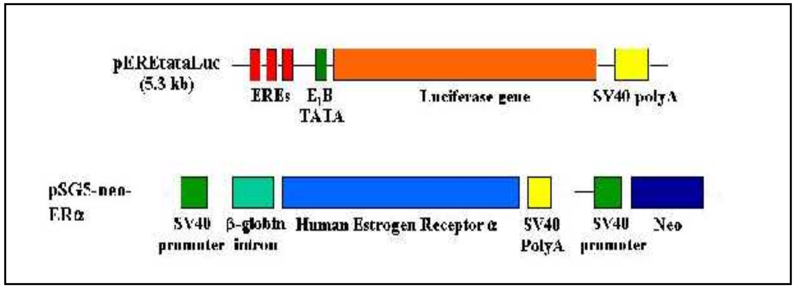
CALUX- biosensors, main marketed products of BDS



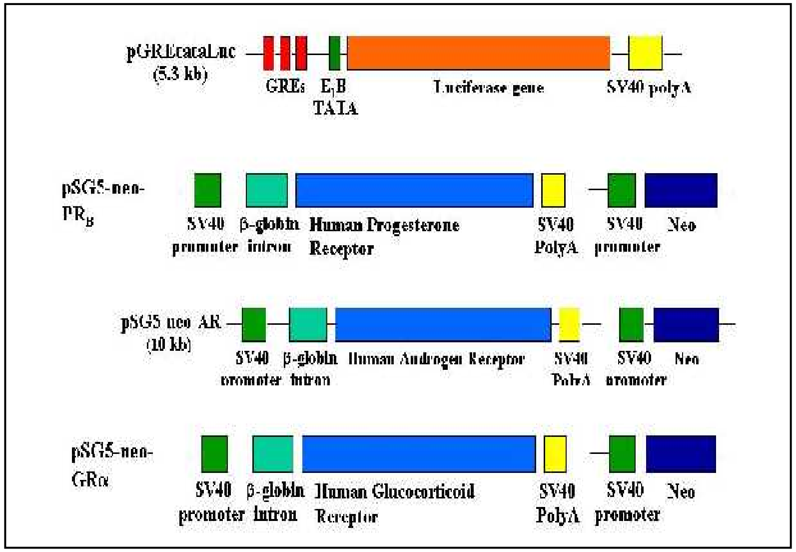


Development of array of steroid reporter gene assays (STERoLUX)

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



ERα and ERβ CALUX®

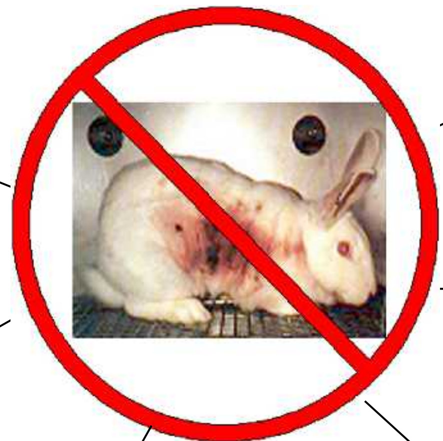


AR, PR, and GR CALUX®

CALUX screening battery

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

Applications CALUX® battery



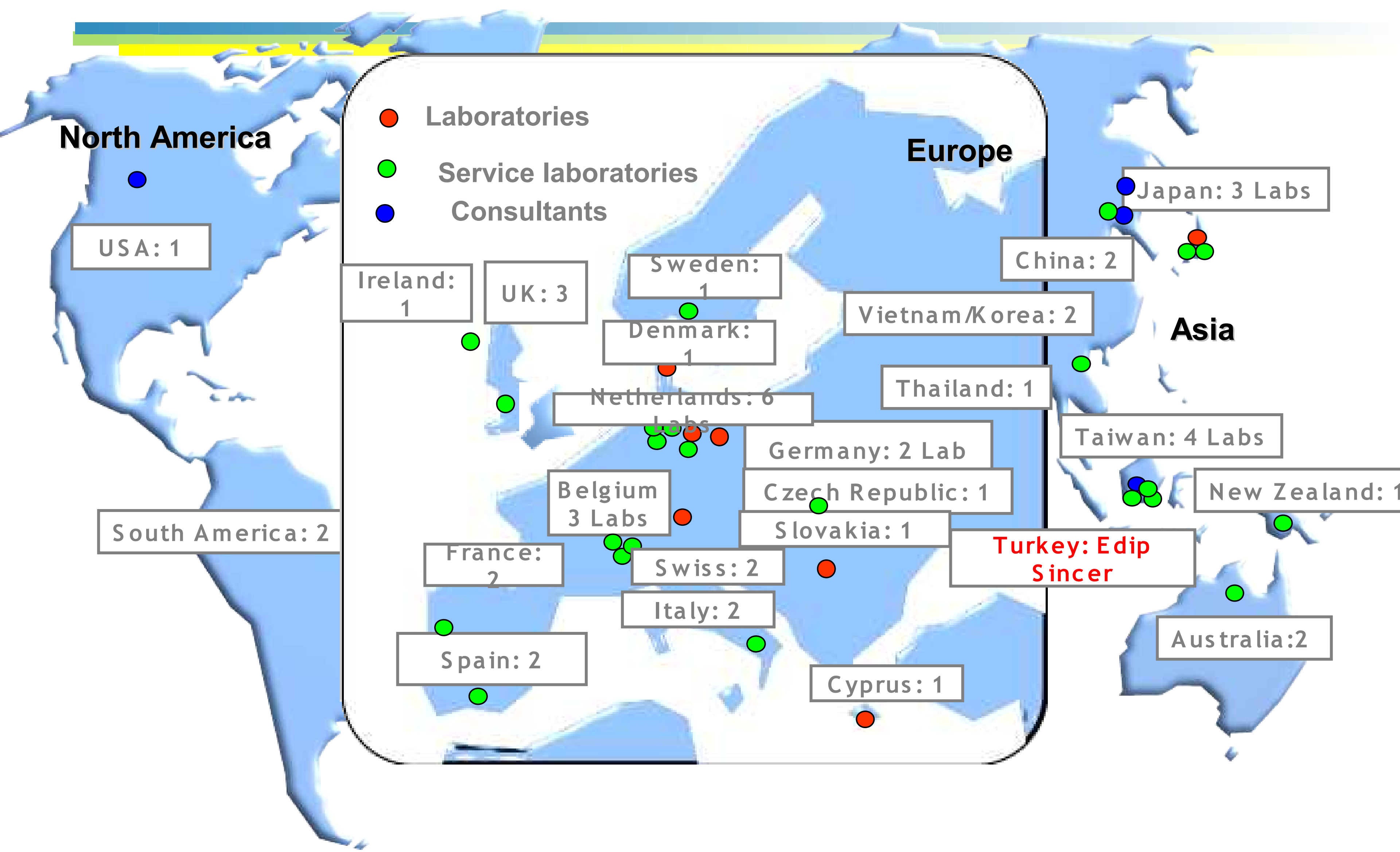
- **Acute**
  KappaB nrf2 cytox p53 p21
- **Endocrine**
  ER AR PR TR GR RAR DR KappaB cytox PPAR
- **Repro**
  ER AR PR TR GR RAR DR KappaB nrf2 cytox PPAR p53 p21
- **Immuno**
  ER AR PR GR RAR DR KappaB nrf2 cytox PPAR
- **Development**
  ER AR TR GR RAR DR KappaB cytox PPAR
- **Muta/carcinogen**
  ER AR PR TR GR RAR DR KappaB nrf2 cytox PPAR p53 p21

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 advarse drug reactions/metabolism
  - Netherlands Toxicogenomics Centre- genomics and non animal testing for drug safety

Name	Topic	CALUX	Period/homepage
FP 6 FaceIt	Oil spill -early warning and toxicity	PAHs	2005-2009 www.unil.ch/face-it
DiscriSet	Hazardous waste	DR	2008-2011 www.vito.be
FP 6 ReProtect	REACH	ER, AR	2006-2009 www.reprotect.eu
FP 6 Techneau	EDC water	CALUX panel	2008-2009 www.techneau.org
FP 6 NewGeneris	Mother-newborn baby health biomarker	DR, ER, AR	2006-2009 www.newgeneris.org
Ecogenomics	Soil quality	CALUX panel	2004-2009 www.ecogenomics.nl
World Anti Doping Agency	Bioassays for anabolic steroids	AR, ER, GR	2004-2008 www.wada-ama.org
Food and Nutrition Delta	Bioassays for health claims functional foods	Various novel	2008-2010 www.foodnutritiondelta.nl
Netherlands Toxicogenomics Centre	REACH-alternative to animal tests	Various novel	2008-2012 www.toxicogenomics.nl
FP7 Metaexplore	New biofunctionals	Various	2009-2013

International acceptance……



Dioxin DR CALUX laboratories approved by BDS

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[1,2] for animal and public health protection. The use of the DR CALUX® 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[3,4]. To ensure the reliability and performance of the DR CALUX® 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® interlaboratory comparison study (BICS 2005) organized by BioDetection Systems BV (BDS) are described. A total of 21 laboratories world wide using the DR CALUX® 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

Type	Purpose	Sector	Duration
Crisis related monitoring	Management of crisis with purpose to resolve swiftly	Import/Export of Food, Feed, Raw materials	Incidental
Source directed Survey	Source identification	Environment, Feed, Food	Incidental/longer term
Background Survey Trend analysis	Background levels reductions	Food, Feed, Raw materials	Long term
Total Dietary Survey (TDS)	Dietary intake estimation	Food, Nutrition, Public Health	Long term
Targeted Survey	Contamination level specific food/feed items	Food, Feed, Nutrition (animal, human), Export	Long term
Human monitoring	Human risk assessment	Public Health	Long term
Environmental Monitoring	Environmental risk evaluation	Environmental Health	Long term

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Crisis related monitoring: Dioxin Feed and Food crises

Brazilian	Citrus pulp 1998
Belgian	Chicken PCB fat 1999
German	Kaolinic clay 1999
Belgian	Cholin chloride 2002
German	Bakery waste 2003
Netherlands	Potato peels/kaolinic clay 2004
Belgium	Gelatin/Hydrochloric acid 2006
Australia	Fish/Home bush bay-2,4,5-T 2006
India	Guar Gum/PCP 2007
Italy	Mozzarella/waste disposal 2008

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

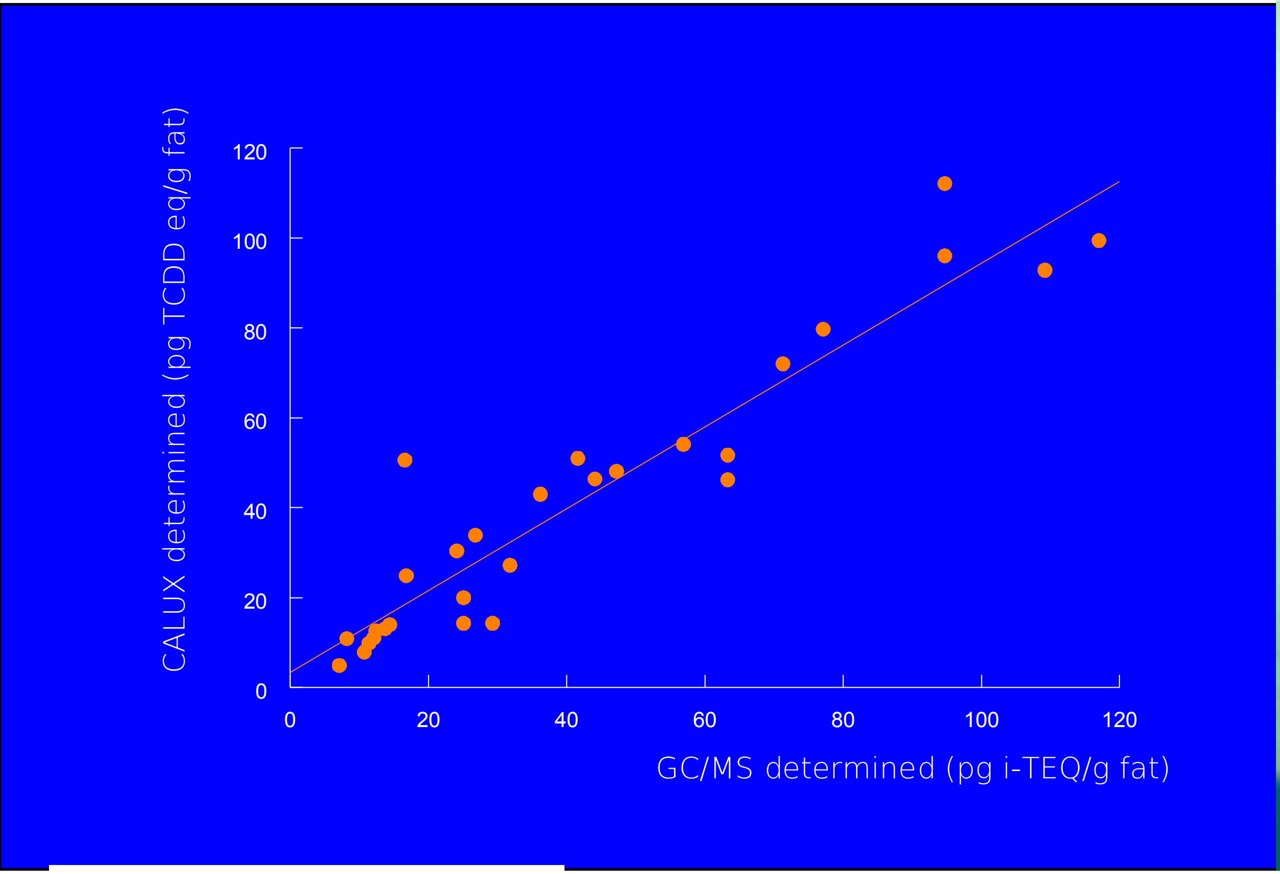
- enabling medium-high frequency analysis at relatively low cost and high capacity

- 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



RIKILT
INSTITUTE OF FOOD SAFETY
WAGENINGEN UR

Crisis related monitoring: Dioxin Feed and Food crises

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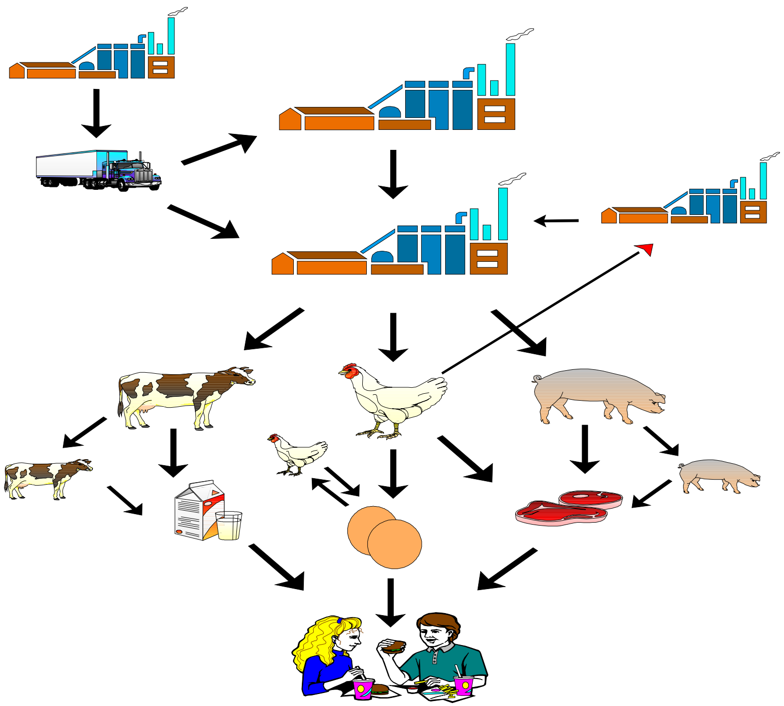
Belgian dioxin chicken crisis 1999

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



Crisis Management by Rapid Methods: Belgium, 1999

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!

Crisis related monitoring: Dioxin Feed and Food crises

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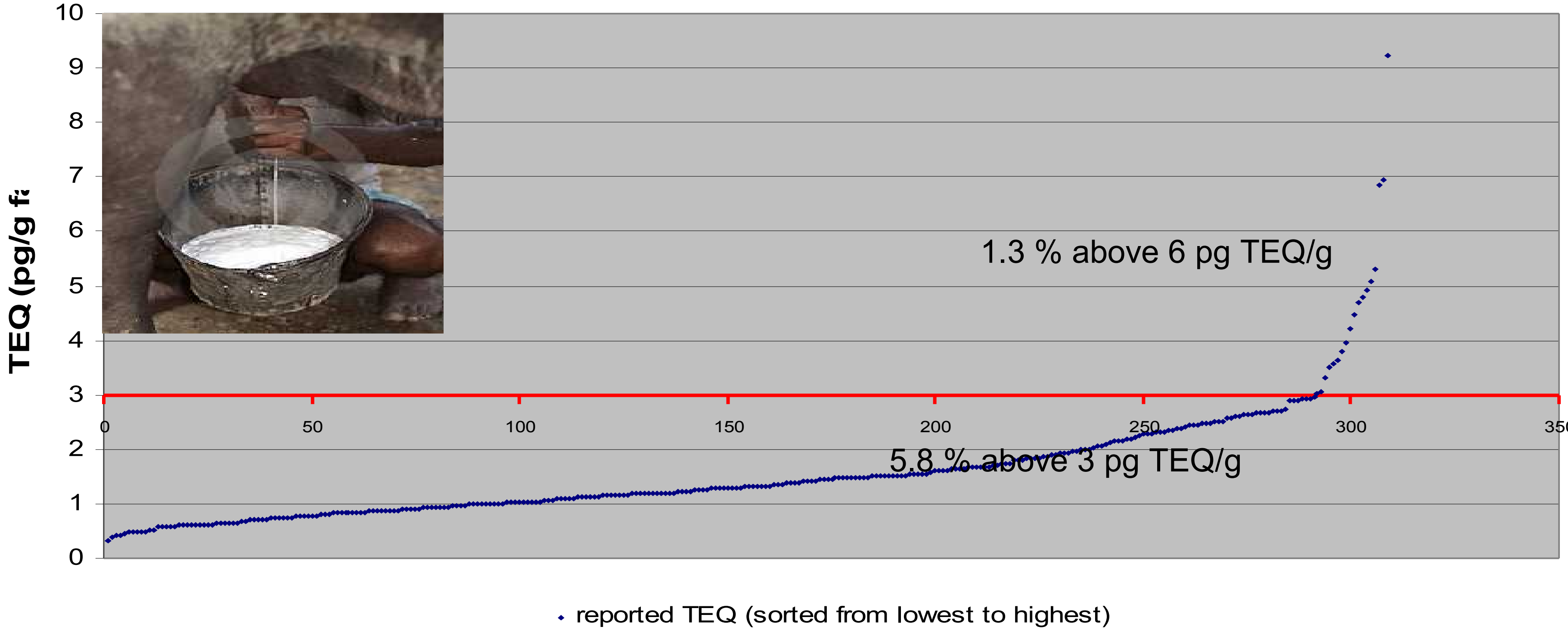
Dutch Dioxin crisis 2004:

Clay effecting potato peelings used in animal feed.

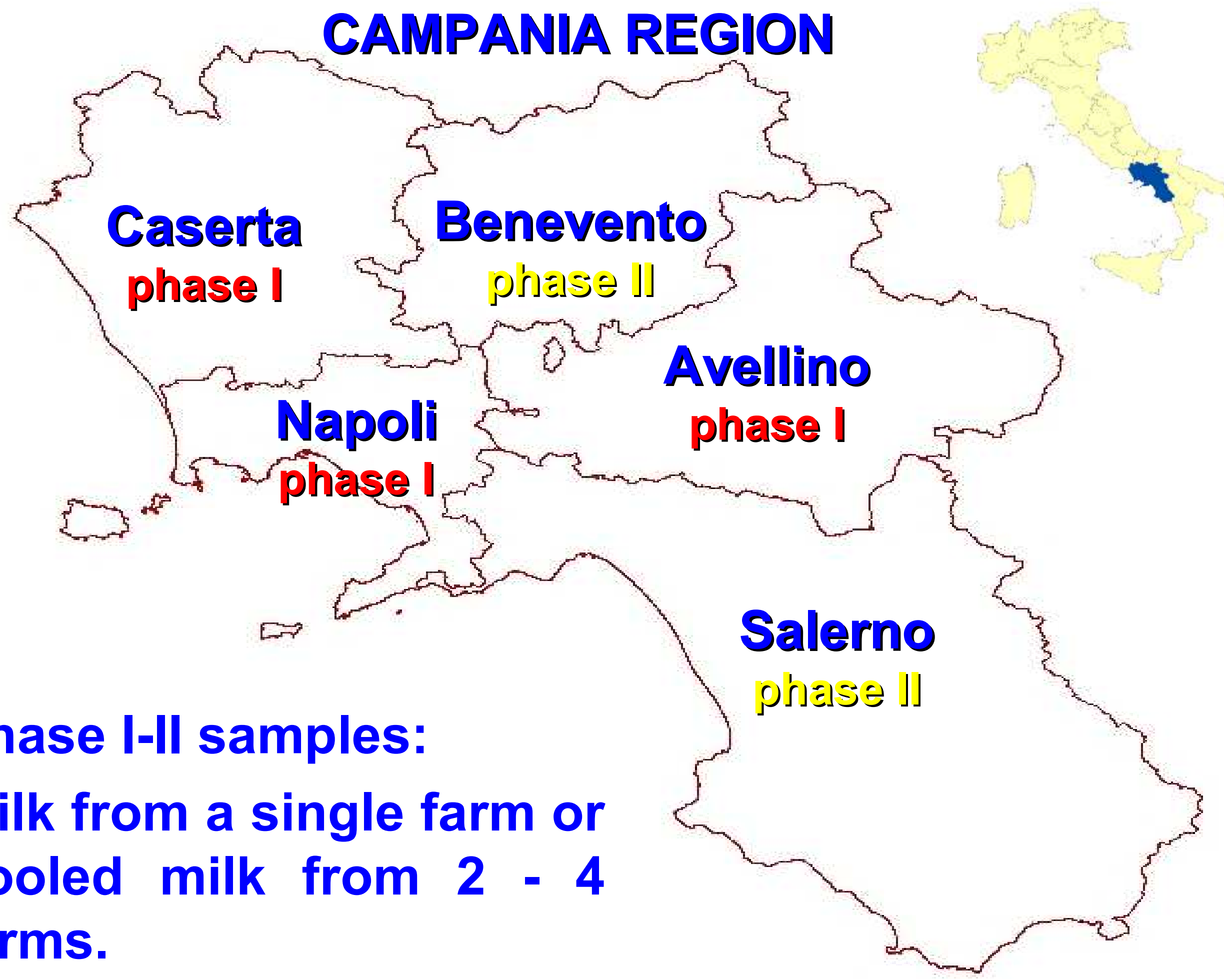
No of samples analyzed during crisis (4 months) several thousands

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



60 % below 1.5 pg TEQ/g

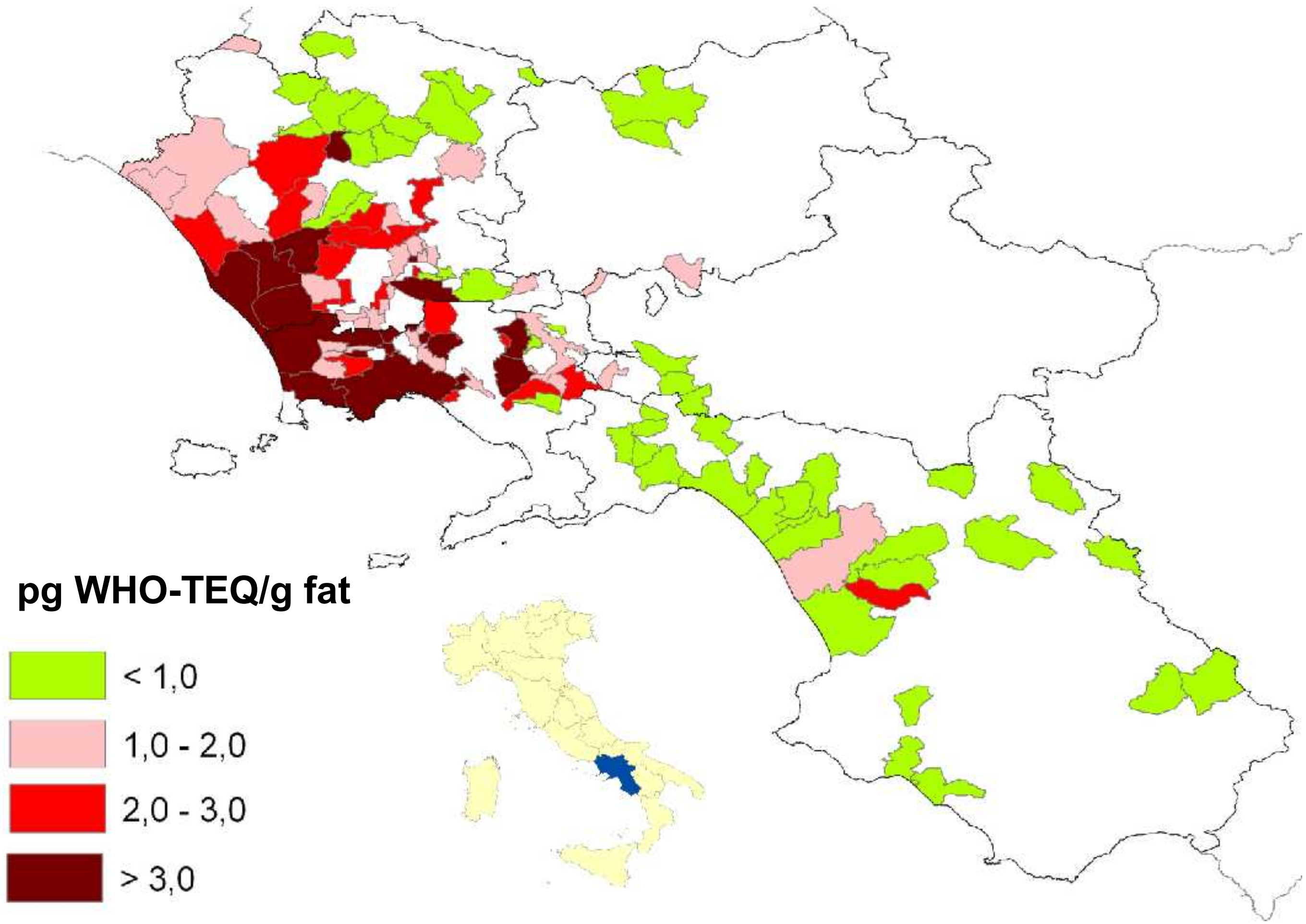
THE MOZZARELLA CHEESE INCIDENT
The monitoring plan design (Scotticini)



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)*



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.**

		a) 2000-03		b) 2004-06		c) 2008	c) Plan 2009
			PCDD/F-TEQ		PCDD/F-TEQ	Media	all IZS
Meat		Sample Nr	Min-Max	Sample Nr	Min-Max	Concern	
	Chicken	74	0.1-1.9	134	0.1-1.0	Mozarella	DR CALUX:
	Bovine	68	0.1-3.7	82	0.1-1.7	in	1500
	Pork	108	0.1-13	146	0.1-0.5	February	
Milk/Dairy	Bovine	123	0.1 - 2.9	183	0.1-0.8	2008	HRGC/MS:
	Sheep/Goat	18	0.1 - 6.2	49	0.1 - 30		1500
	Buffalo	0	nd	50	0.1 - 17	Ca. 200	
Eggs	Chicken	87	0.1-1.6	214	0.1 - 88	samples	
Fish	Trout	34	0.1-1.1	56	0.1-0.2		
	Eel	8	0.1-1.7	10	0.1-0.8	analyzed	
	Seabass	16	0.1-1.7	17	0.1-0.8	by DR	
	Salmon	0	nd	6	0.1-0.3	CALUX	
	Others	7	0.1-0.6	0	nd		
Samples/year ca.:		180		310		1200	3000

The Eggs contamination case (Scotticini, 2004) 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.

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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)**

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Strategy to reduce presence of dioxins and dl-PCBs in feed and food

- 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

Product	Tolerance limit	Tolerance limit	Action limit	Action limit
	dioxins	dioxins and dl-PCBs	dioxins	Dl-PCBs
Milk fat	3	6	2	2
Poultry fat	2	4	1.5	1.5
Beef fat	3	4.5	1.5	1
Pig fat	1	1.5	0.5	0.5
Eggs	3	3	2	2
Fish	4	8	3	3
Vegetable oil	0.75	1.5	0.5	0.5
Feed	0.75	1.25	0.5	0.35

Food Monitoring

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).

Product, including also derived products	Aquaculture (*)	Wild caught fish (*)	Meat (**)	Milk (***)	Eggs (****)	Other (*****)	Total
No of samples	250	483	500	250	250	267	2 000
Belgium	4	8	18	8	7	7	52
Denmark	4	20	14	7	4	6	55
Germany	16	28	55	34	25	36	194
Greece	6	8	14	8	4	7	47
Spain	26	36	36	13	24	21	156
France	25	30	55	28	28	27	193
Ireland	8	15	15	7	5	4	54
Italy	22	24	46	20	26	26	164
Luxembourg	2	3	6	3	3	3	20
Netherlands	7	18	26	13	20	8	92
Austria	3	3	15	8	6	7	43
Portugal	4	12	12	6	5	6	45
Finland	4	10	10	6	4	6	40
Sweden	4	12	10	6	4	6	42
United Kingdom	15	30	40	19	20	20	144
Czech republic	6	3	11	5	5	5	35
Estonia	2	6	7	3	2	4	24
Cyprus	2	6	4	3	2	3	20
Latvia	2	6	7	3	2	4	24
Lithuania	2	6	7	3	2	4	24
Hungary	3	3	11	5	10	5	37
Malta	2	3	4	3	2	3	17
Poland	10	18	25	13	16	20	102
Slovenia	2	3	7	3	2	4	21
Slovakia	2	3	7	3	2	4	21
Bulgaria	4	3	9	5	5	4	30
Romania	6	3	11	9	9	10	48
Iceland	3	69	7	3	2	3	87
Norway	54	94	11	3	4	4	170
Total	250	483	500	250	250	267	2 000

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.

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.

Total number samples recommended for each country		Feed materials, additives, premixtures											Compound feedingstuffs					
		Plant origin							Animal origin			Total	Terrestrial animals				Fish	Total
Country (*)	Number	Cereals, grains, their products and by-products	Oil seeds oil fruits, their products and by-products/legume seeds, their products and by-products	Forages and roughage	Other feed materials of plant origin	Minerals	Trace elements, binders, anti-caking agents	Premixtures — all species	Animal fat/animal products (including milk powder and egg products)	Fish oil	Fish meal	Number	Cattle	Pigs	Poultry	Other (rabbit, horse, pet food)	Fish	Number
Belgium	60	5	5	5	3	3	3	3	4	3	3	37	4	10	5	2	2	23
Denmark	107	5	5	5	3	3	3	4	3	24	23	78	4	10	3	2	10	29
Germany	163	20	12	11	9	9	9	8	10	3	3	94	24	19	14	4	8	69
Greece	53	5	5	3	2	2	2	3	3	4	3	32	2	2	2	1	14	21
Spain	135	8	6	5	7	8	8	8	6	5	9	70	12	21	14	8	10	65
France	232	28	19	28	11	11	11	12	7	4	5	136	15	19	32	15	15	96
Ireland	56	5	3	5	2	3	3	3	3	3	3	33	7	3	3	3	5	21
Italy	117	10	7	12	5	5	5	7	5	4	3	63	12	6	14	7	15	54
Luxembourg	33	3	3	3	2	1	1	2	2	1	1	19	3	3	3	2	3	14
Netherlands	111	5	5	5	7	8	8	7	5	3	3	56	14	19	13	6	3	55
Austria	47	5	5	5	2	2	2	3	3	3	3	33	3	3	3	2	3	14
Portugal	50	3	5	5	2	3	3	3	3	3	3	33	4	3	5	2	3	17
Finland	48	5	3	5	2	3	3	3	3	3	3	33	3	3	3	2	4	15
Sweden	49	5	3	6	2	3	3	3	3	3	3	34	4	3	3	2	3	15
United Kingdom	158	10	10	10	6	6	6	10	4	10	8	80	15	7	13	10	33	78
Total EU	1 417	122	96	113	65	70	70	79	64	76	76	831	126	131	130	68	131	586
Iceland	67	3	3	3	2	1	1	2	3	19	16	53	3	3	3	2	3	14
Norway	127	5	5	5	3	3	3	5	3	13	15	60	3	3	3	2	56	67
Total EEA	1 611	130	104	121	70	74	74	86	70	108	107	944	132	137	136	72	190	667

(*) 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.

Guideline 2004/704/EC

- 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.

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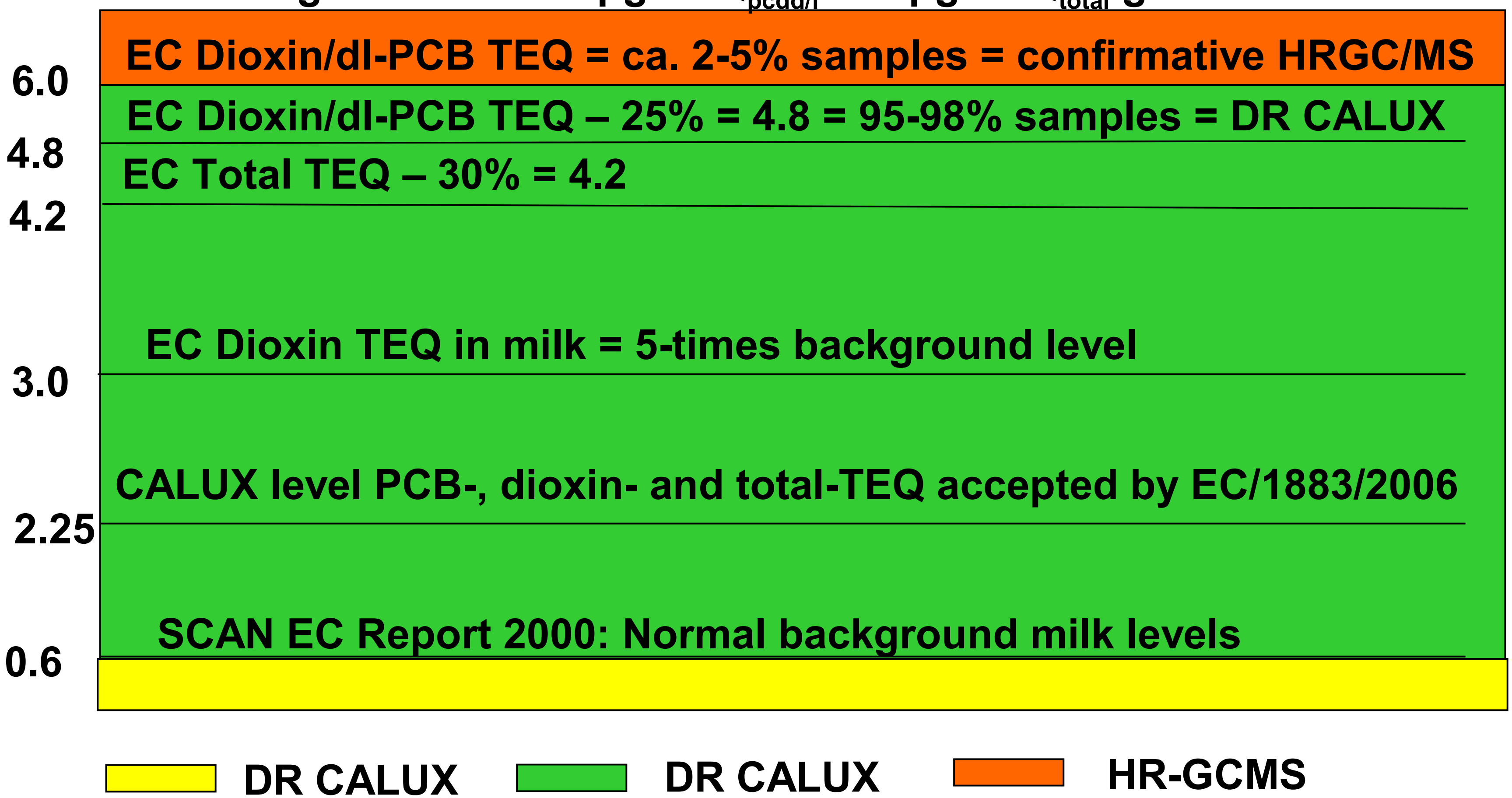
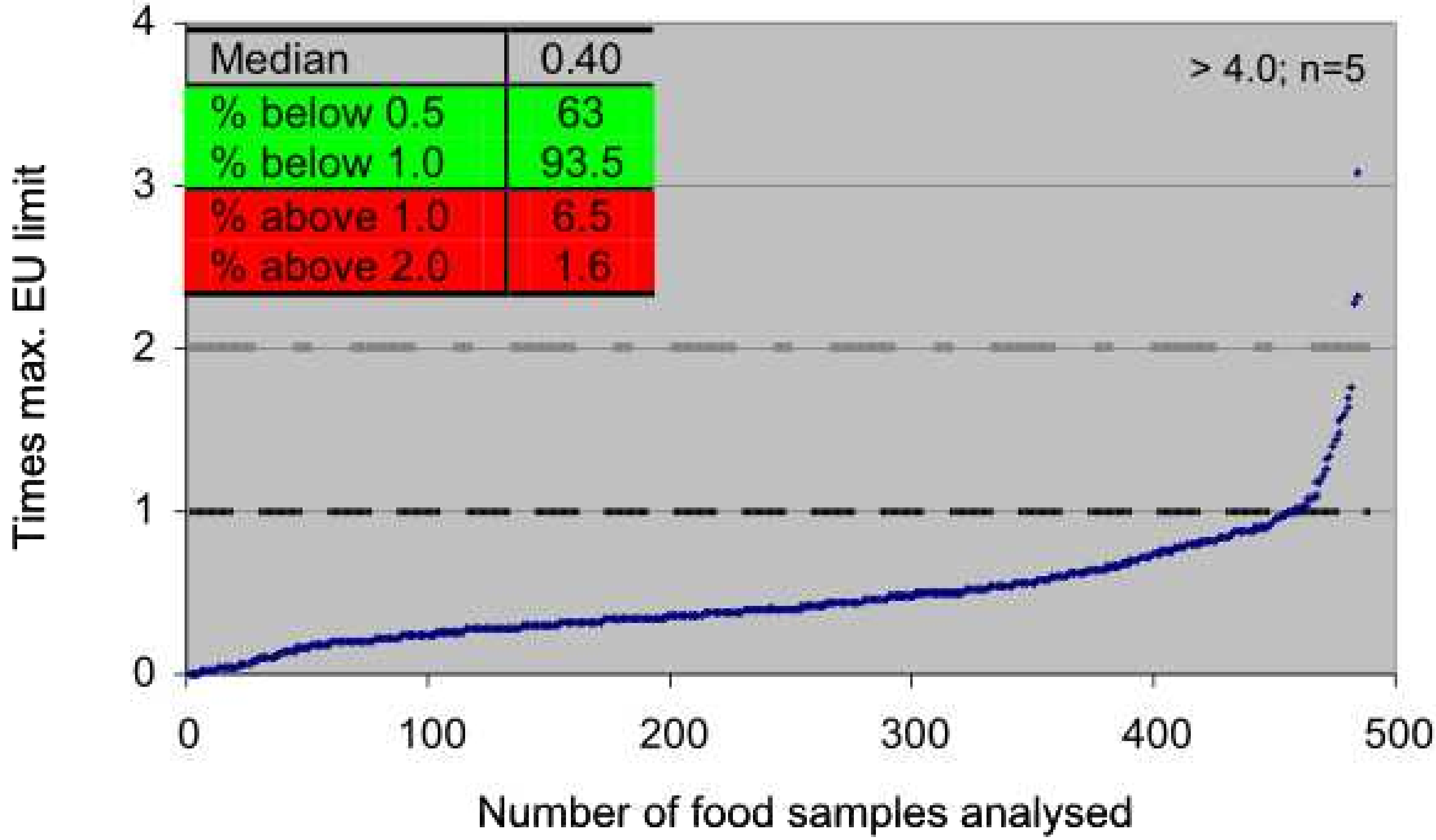
Where is the application zone of CALUX with respect to EU limit values for feed and food

Example: National Monitoring Plans for MILK :

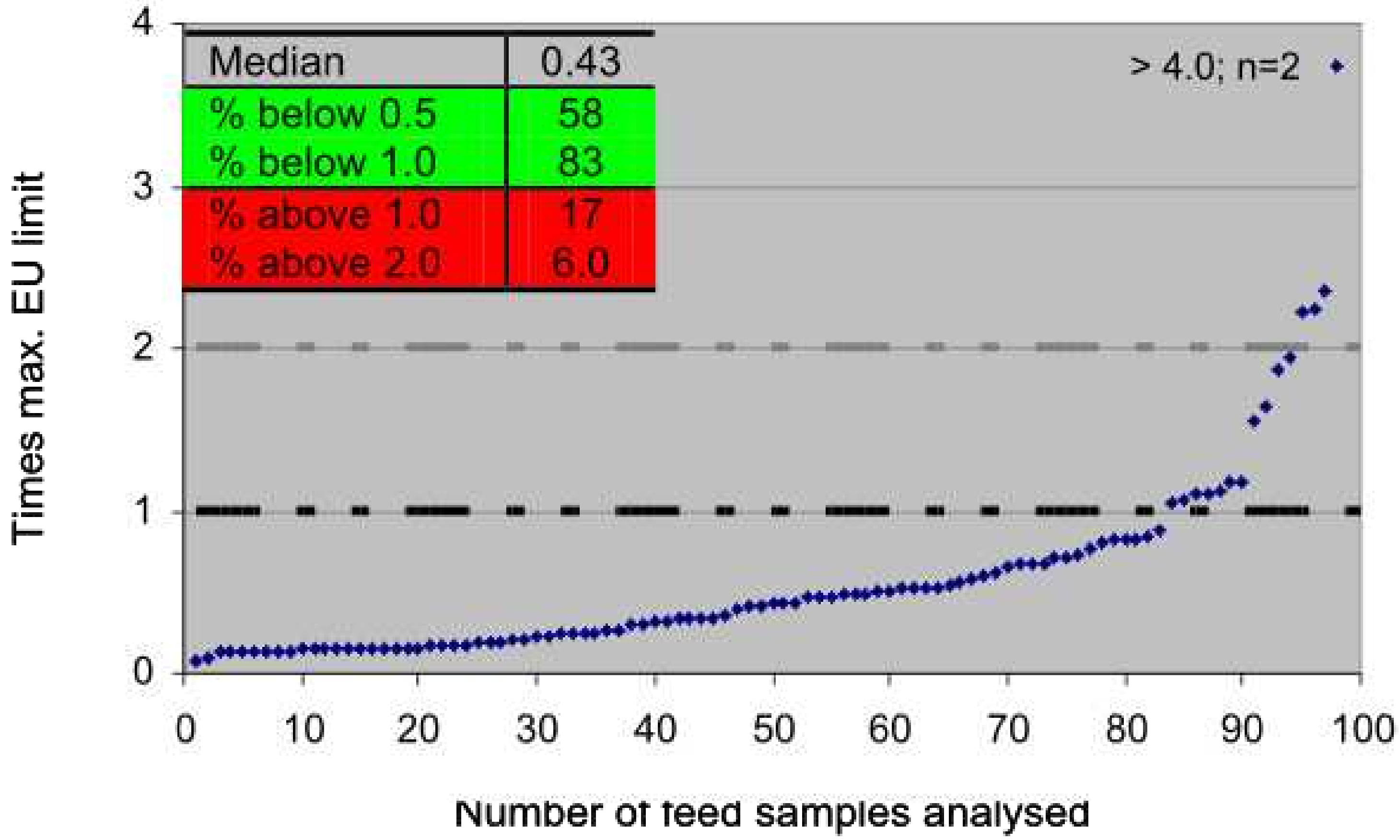
regulated level 3 pg $TEQ_{pcdd/f}$ or 6pg TEQ_{total}/g fat



Background survey: Distribution of DR CALUX® TEQ in food samples (n=490) EU countries

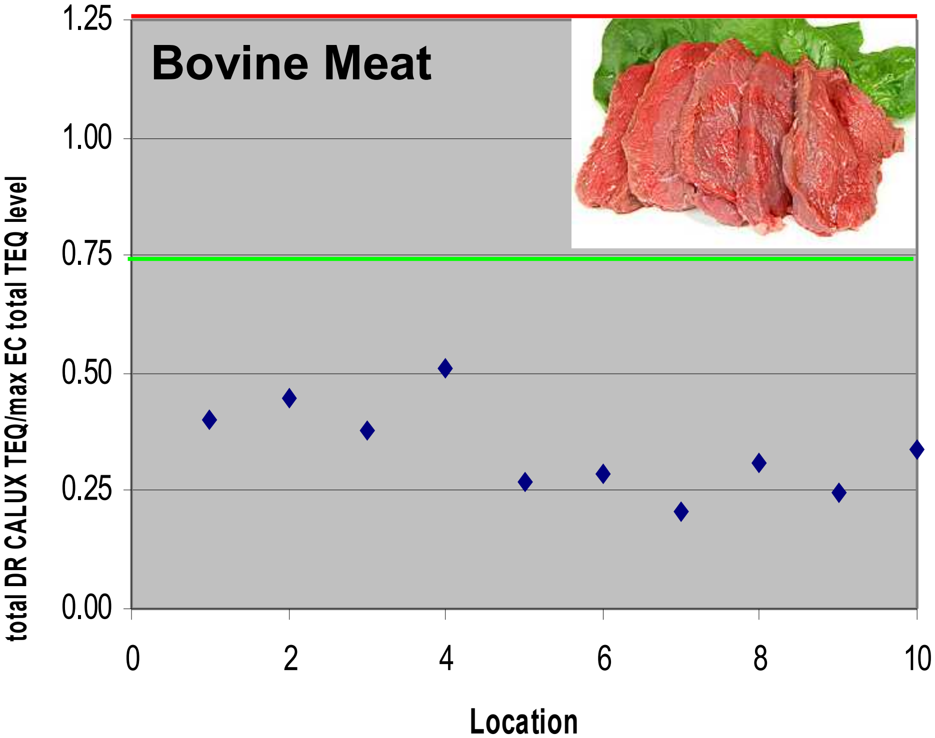


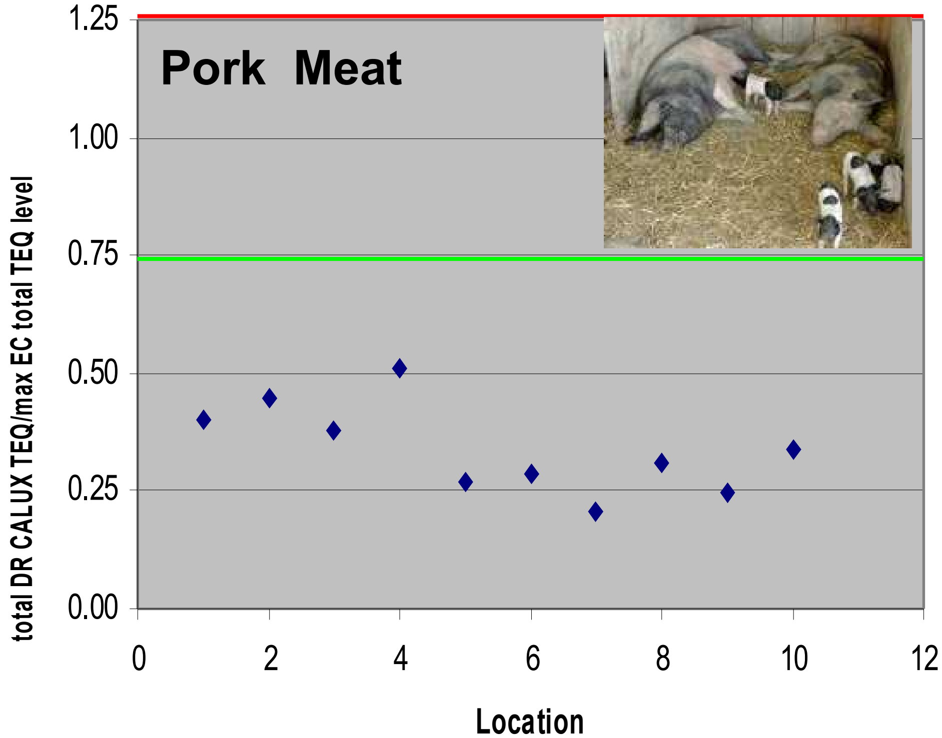
Background survey: Distribution of DR CALUX® TEQ levels in feed samples (n=100) EU countries



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

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





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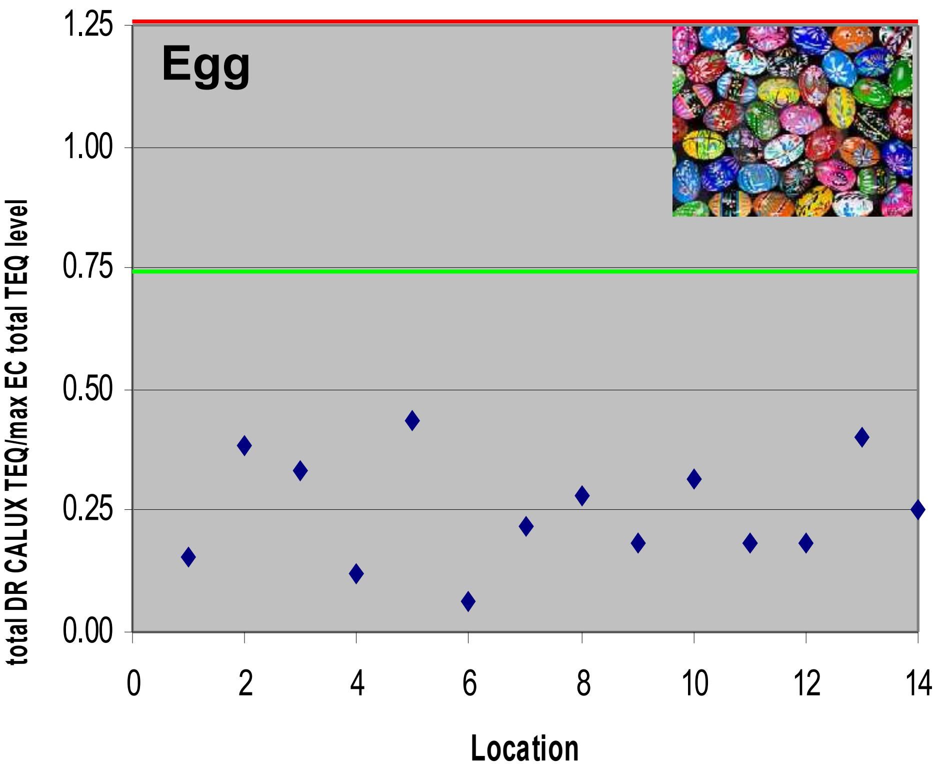
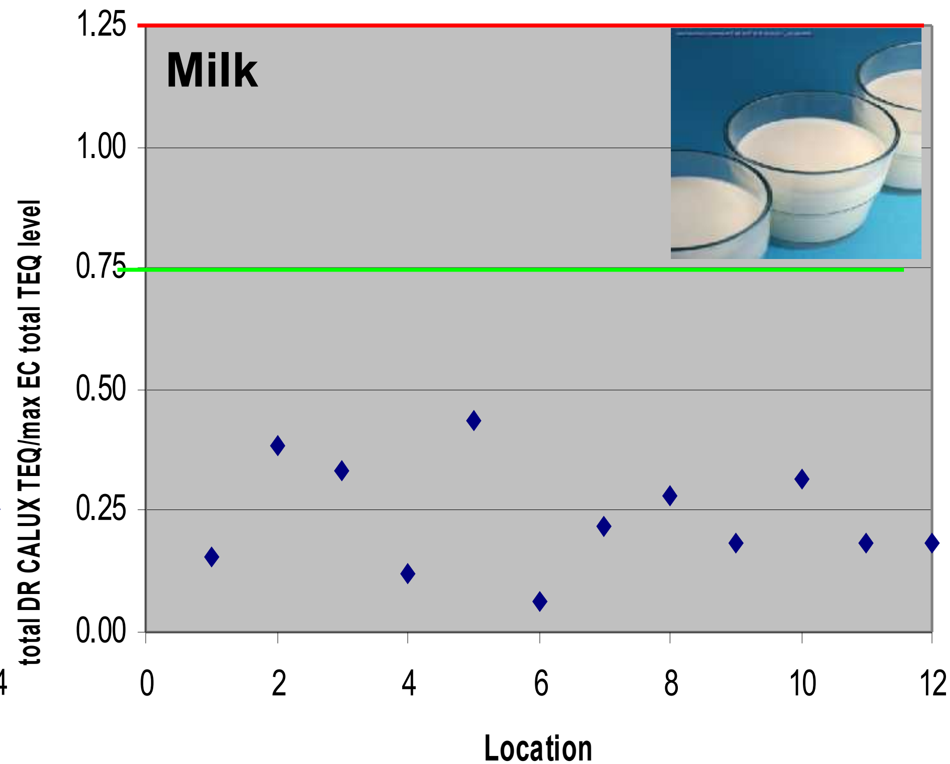
Ratio: Total DR CALUX TEQ vs. accepted European Total-TEQ





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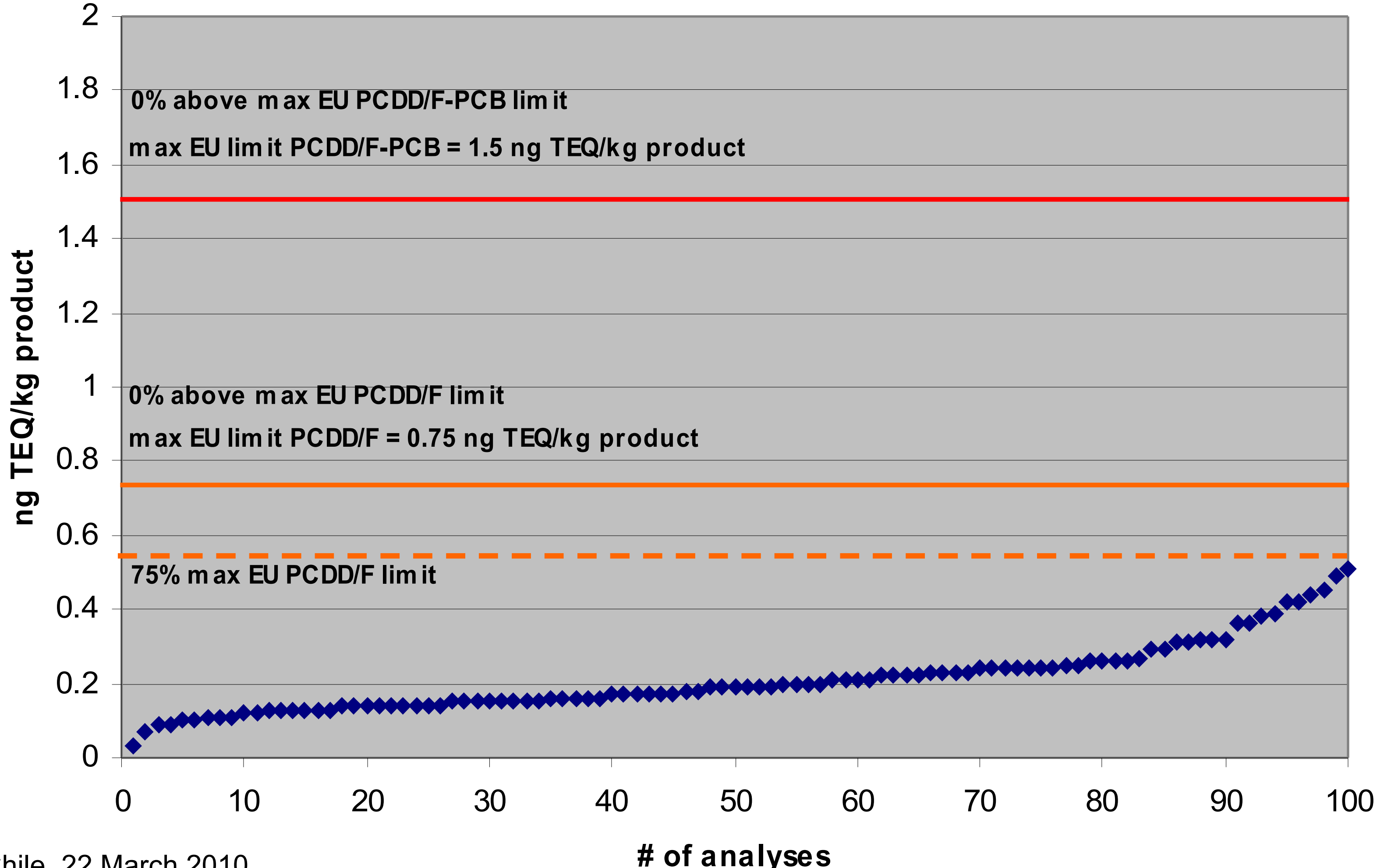
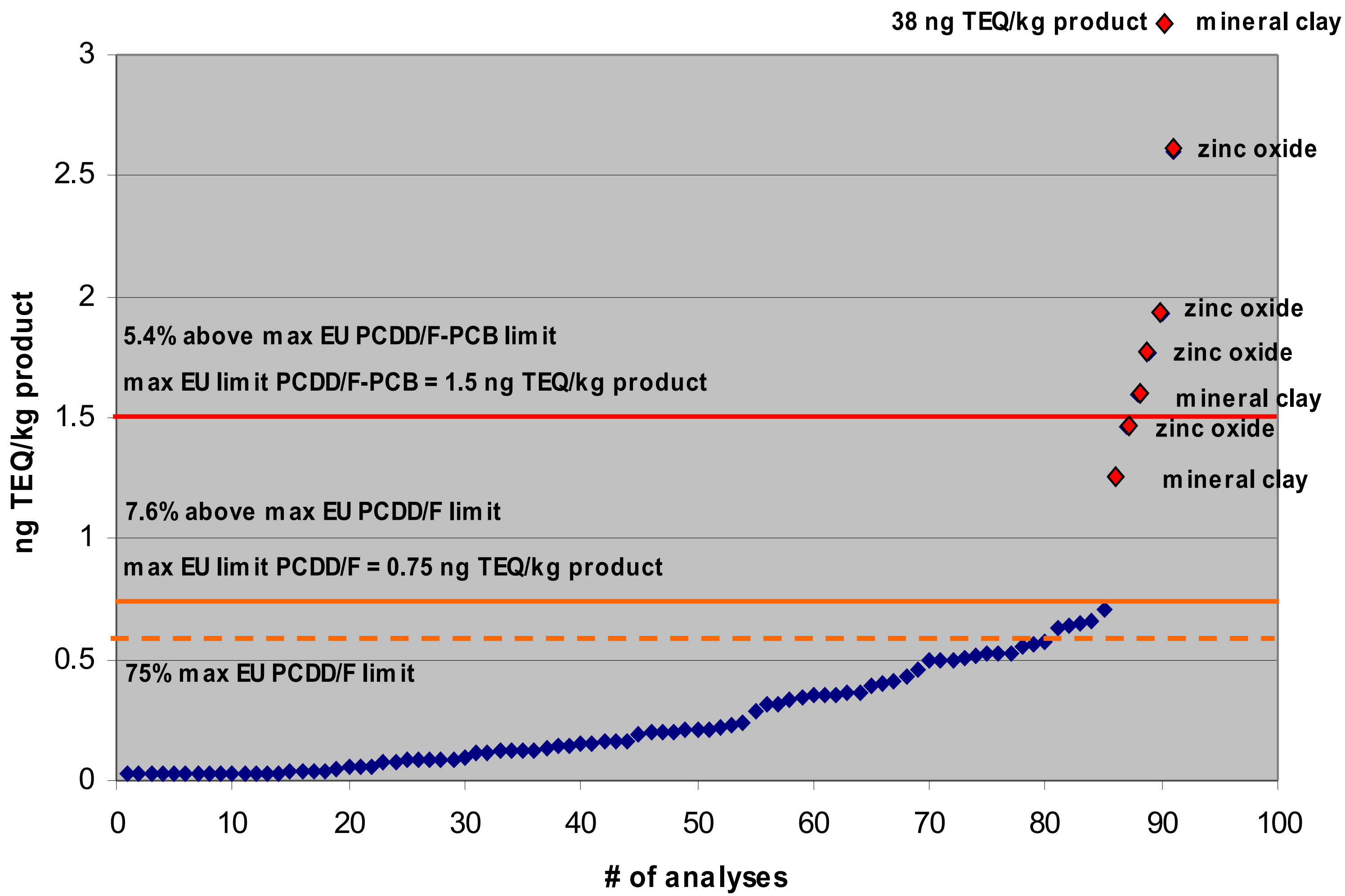
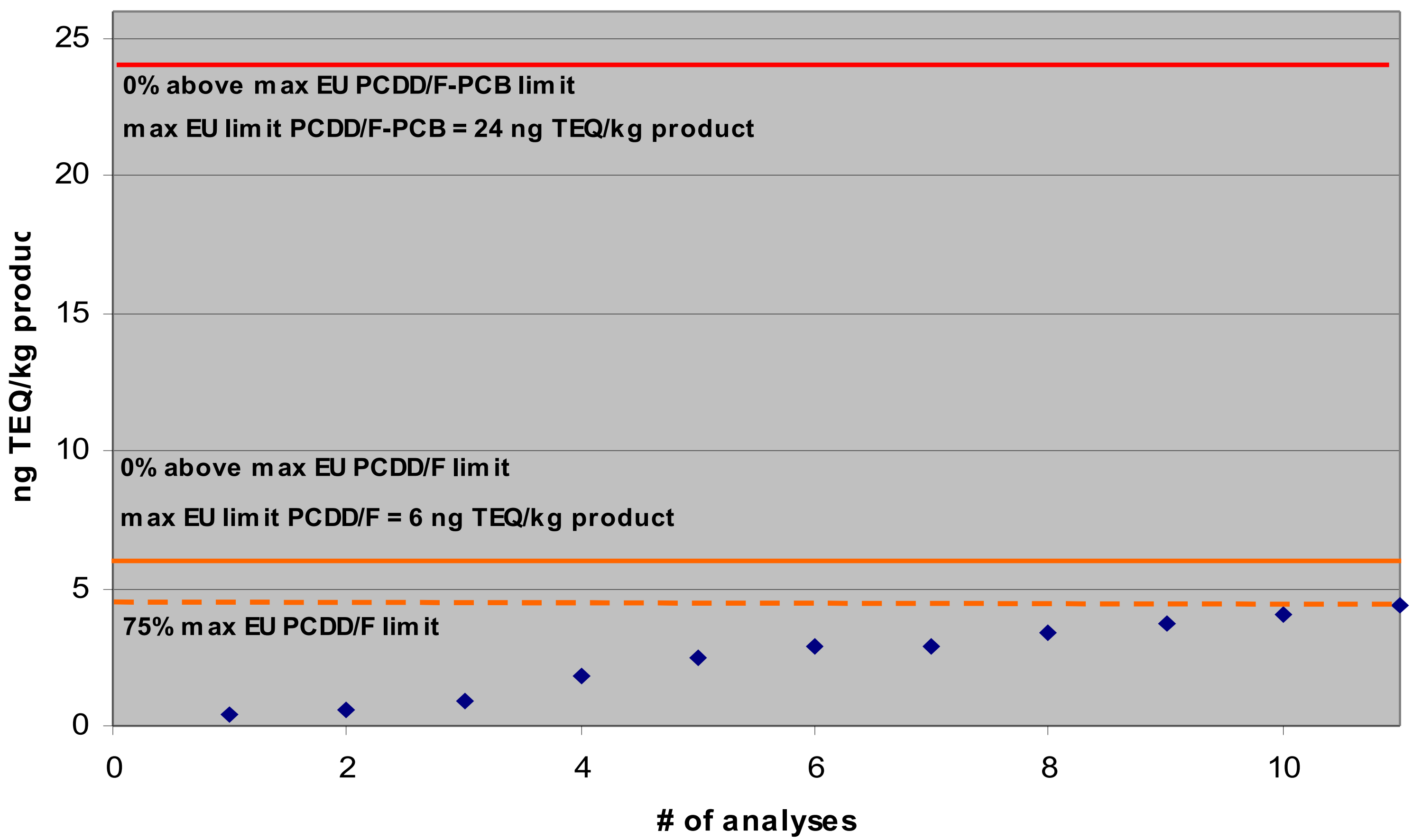
Total DR CALUX® distribution for FEED samples, 2009 Asprocer Program, Chile



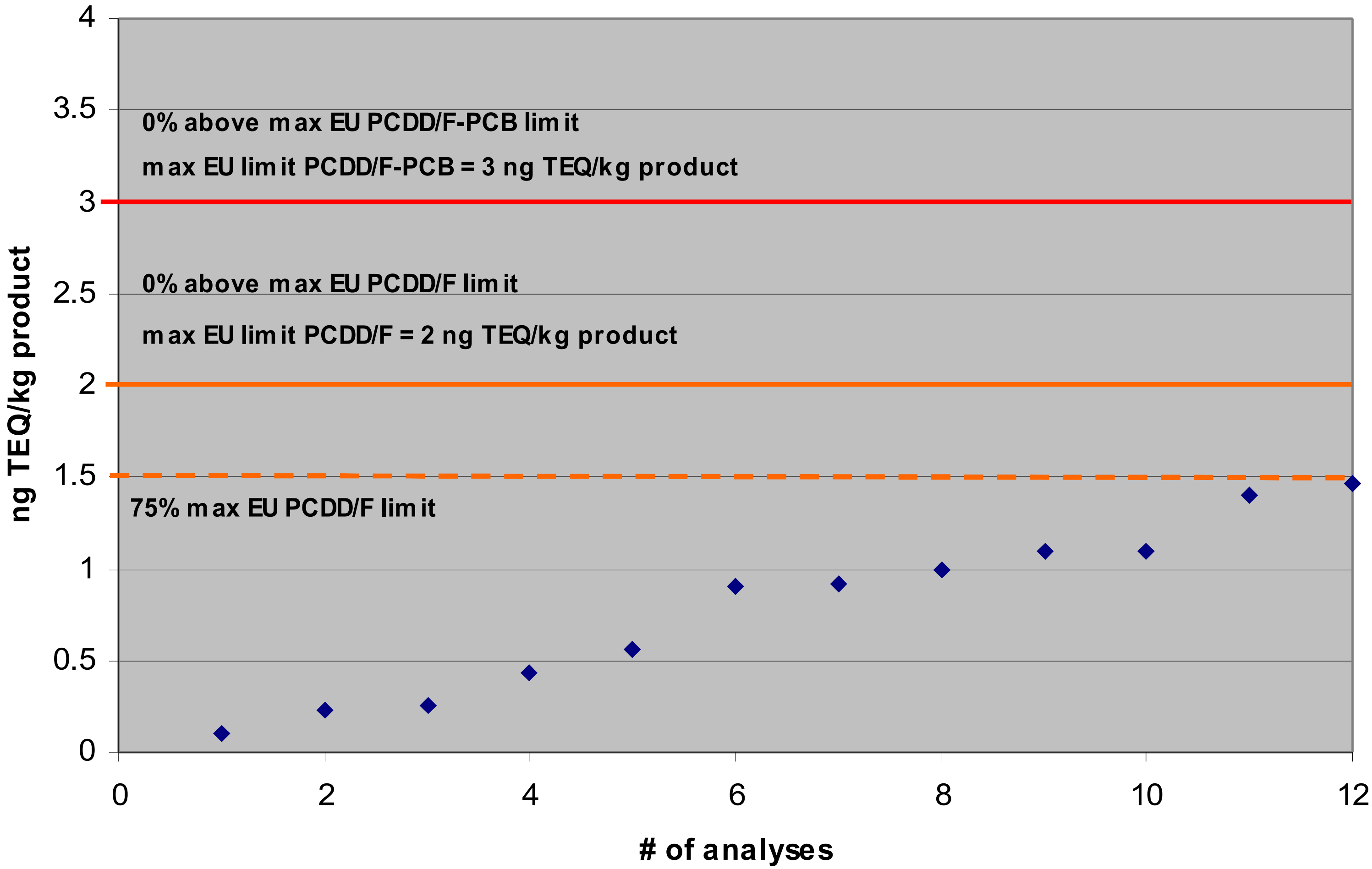
Total DR CALUX® distribution for MINERAL samples, 2009 Asprocer Program, Chile



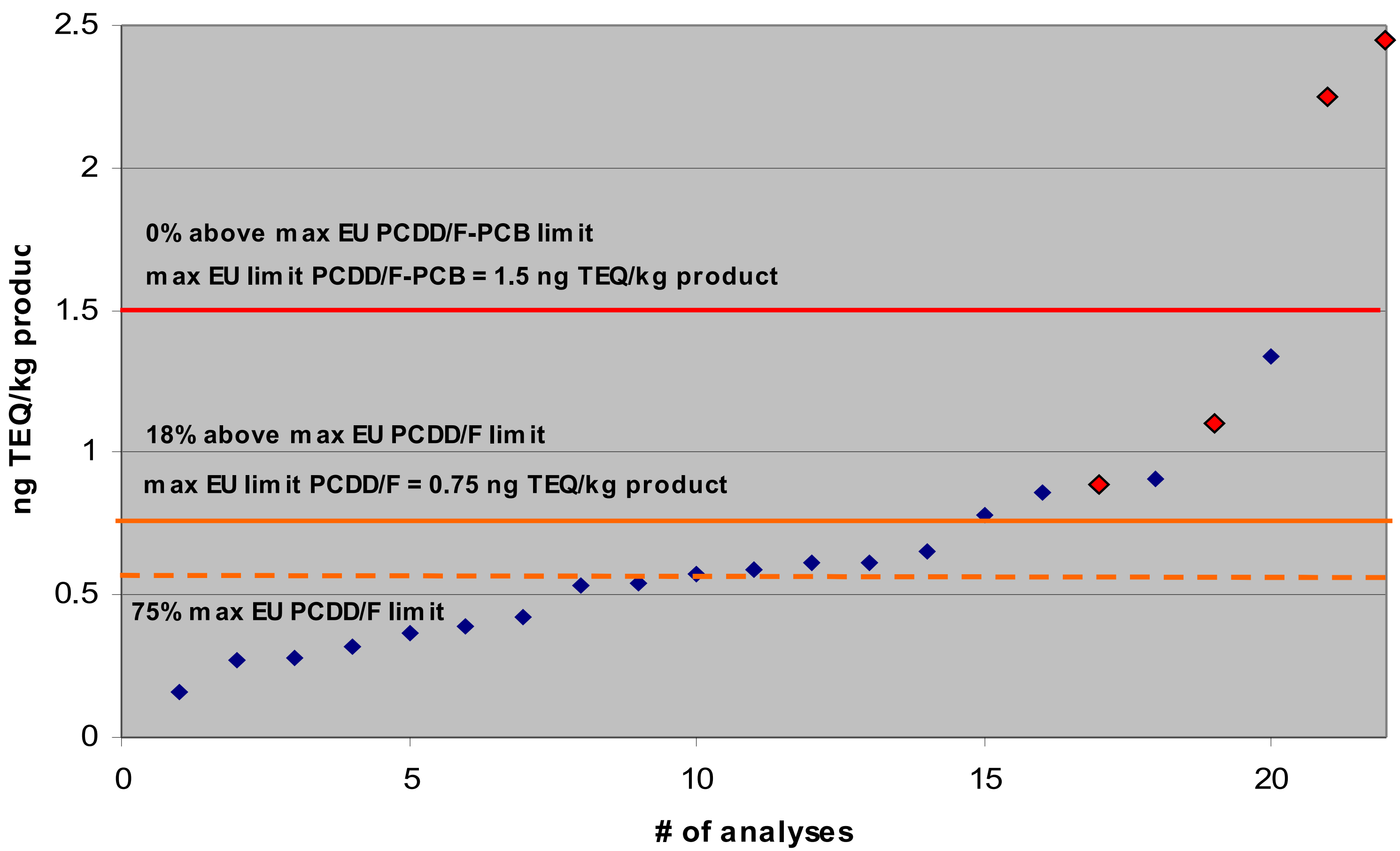
Total DR CALUX® distribution for FISH OIL samples, 2009 Asprocer Program, Chile



Total DR CALUX® distribution for ANIMAL OIL samples, 2009 Asprocer Program, Chile



Total DR CALUX® distribution for VEGATABLE OIL samples, 2009 Asprocer Program, Chile



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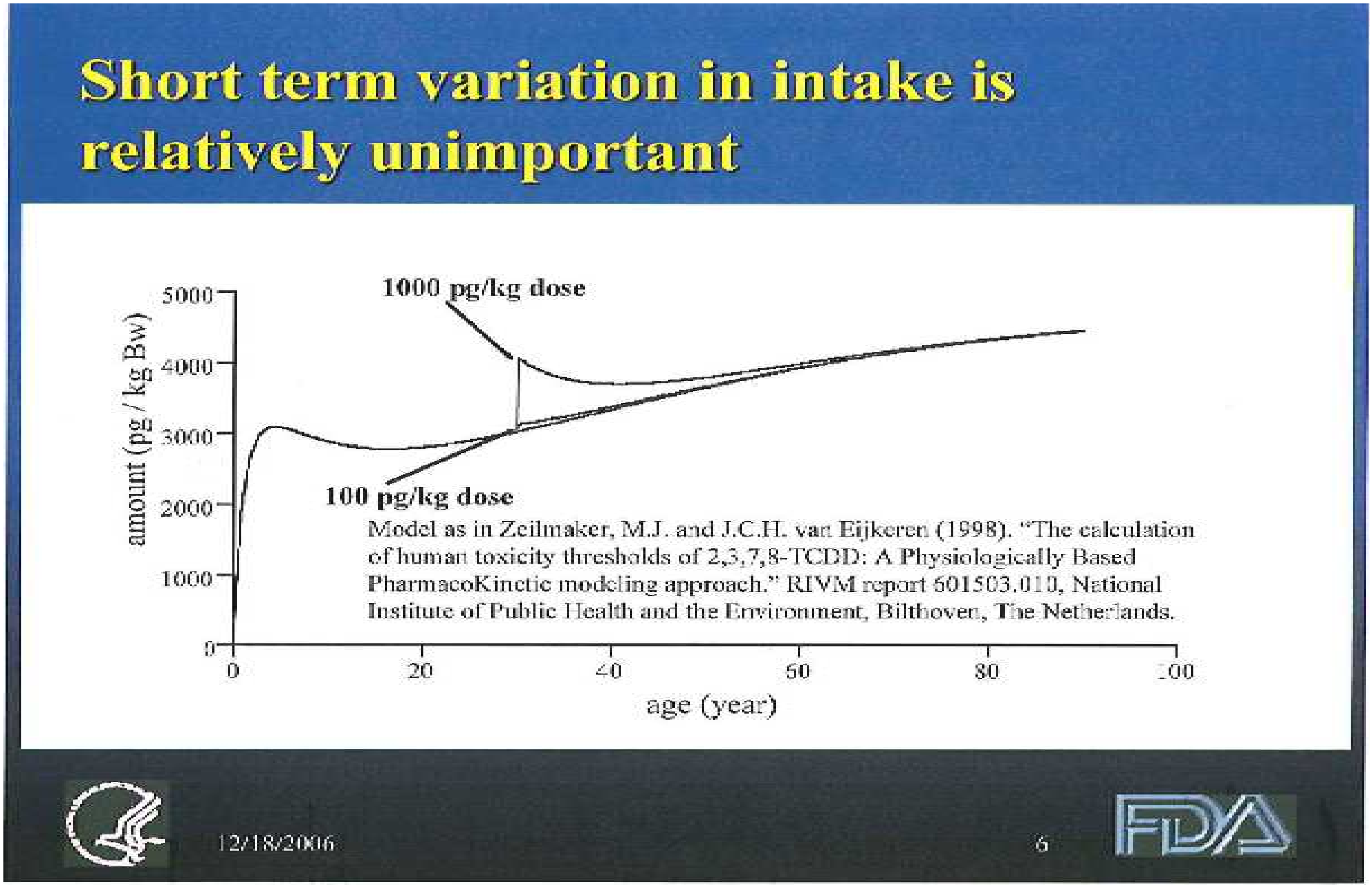
Human exposure to dioxins and dl-PCBs

- 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

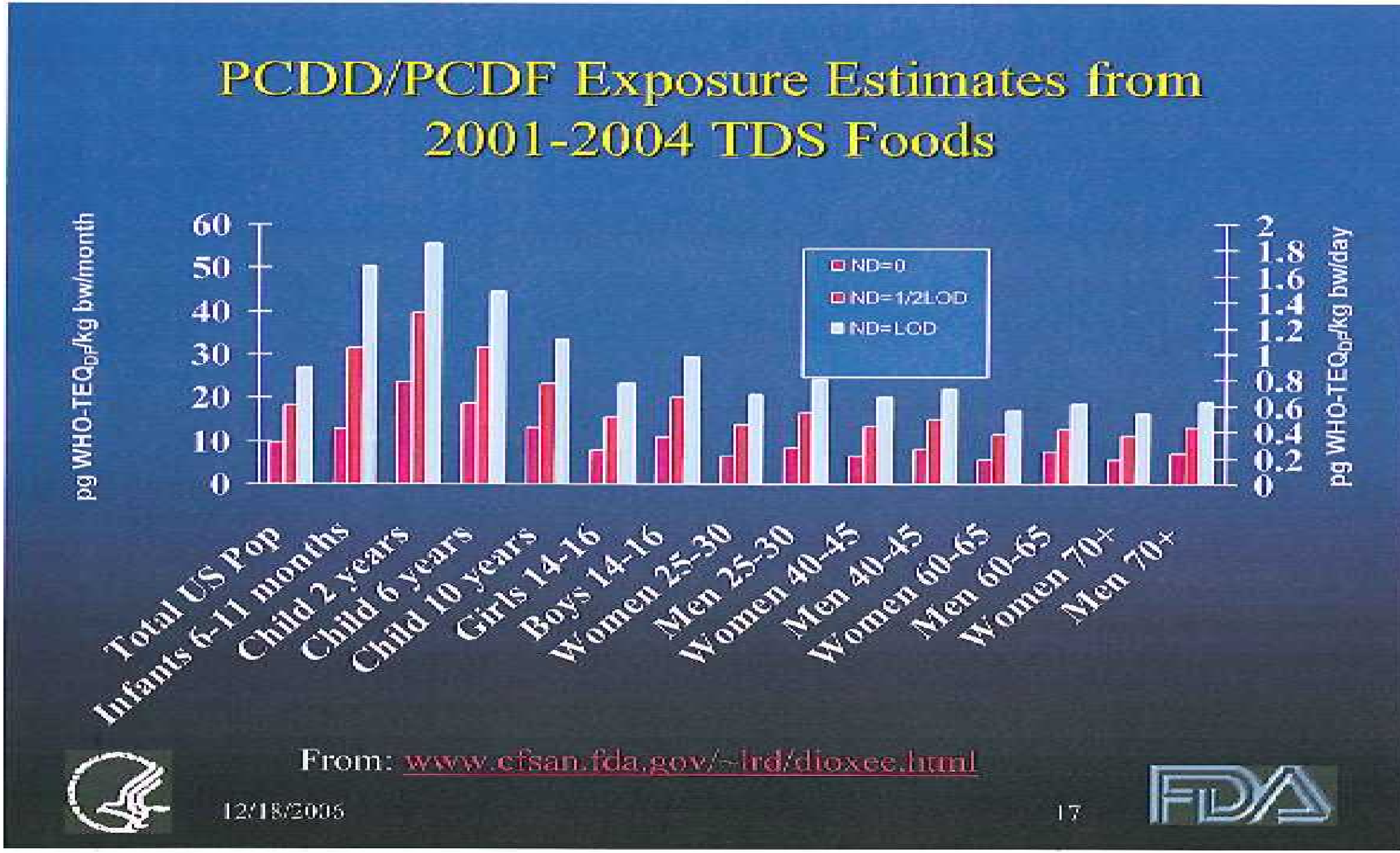
Tolerable human intake of dioxins

- **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



Human intake estimates based on TDS foods intake



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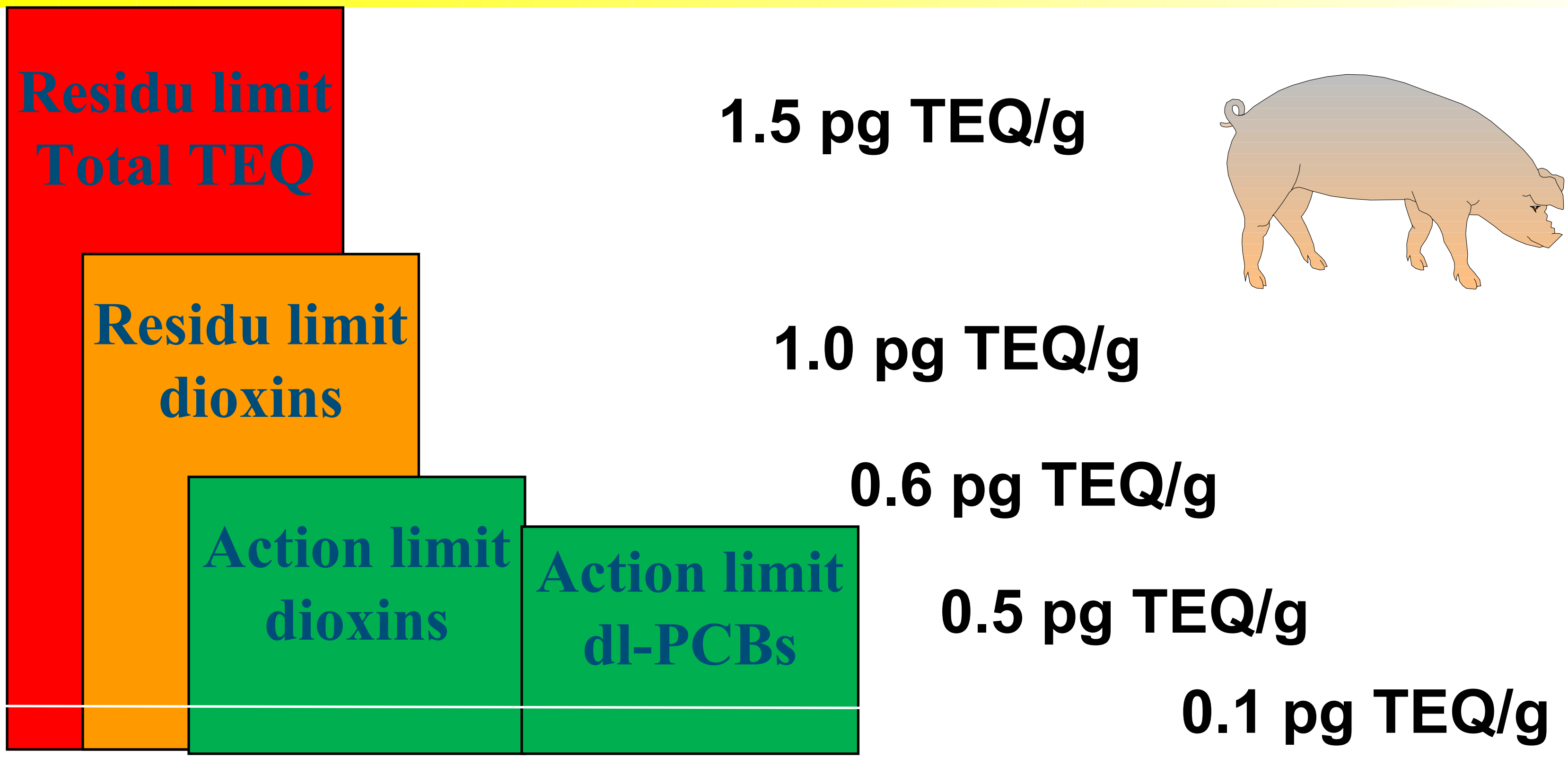
Target limits, action limits and residu limits



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?**

BioDetectors Workshop
in Amsterdam in September/October 2010