Risks of dioxins and dioxin-like PCBs

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RIKILT Wageningen University and Research

Institute for Food Safety

- Works primarily for:
  - Ministry of Agriculture, Nature and Food Quality, and Ministry of Public Health
  - NVWA (food inspection)
  - EU, EFSA
- Expertise
  - Chemical analysis
  - Bioassays/immunoassays
  - Toxicological research
  - Advice (with RIVM)

RIKILT Wageningen University and Research

Institute for Food Safety

- National Reference laboratory for many compounds
  - Including dioxins and PCBs, heavy metals, mycotoxins, plant toxins, marine toxins
- European Reference laboratory for
  - Hormones
  - Mycotoxins and plant toxins
- Emergency service
  - Involved in major incidents

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Dioxins in the news

Egg scare shuts 4700 farms in Germany

January 8, 2011.

Source: The Sydney Morning Herald

AllAbouFeed.net

Dioxin cause in German beet pulp found

Animal feed news

18 Nov 2011

Delhaize withdraws organic eggs

Fri 26/08/2011 - 12:11

Source: FlanderNews.be

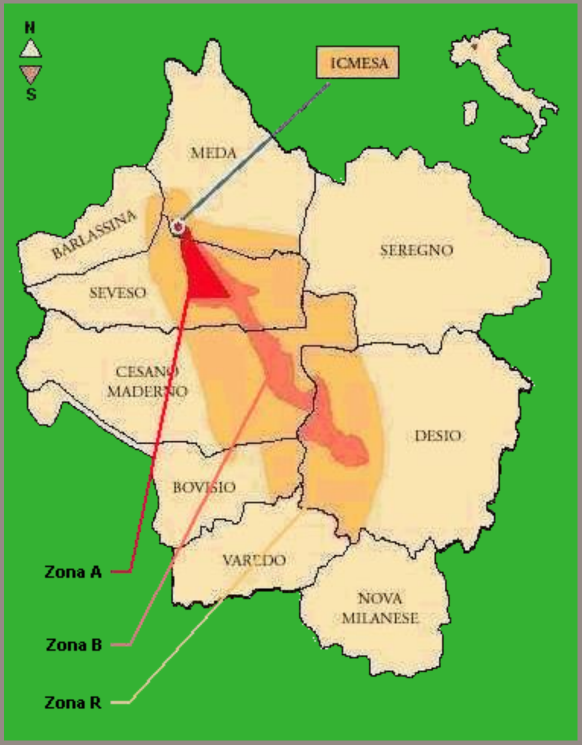
Adverse health effects of dioxins

Victor Yushchenko: poisoned with a few mg of TCDD (2004)
Appearance of chloracne pointed to dioxins

Seveso 1976

- ICMESA chemical plant near 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
- Consequences
  - First signs were yellow leaves, dead chickens and rabbits
  - Company initially did not warn people
  - Large scale evacuation most contaminated areas

Seveso, first effects in humans



Zone	Subjects 3–14 Yrs.	Chloracne cases	Percent
A total	214	42	19.6
A-max(a)	54	26	48.1
B	1,468	8	0.5
R	8,680	63	0.7
R Polo(b)	750	19	2.5
Outside	48,263	51	0.1

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

Health effects Seveso

- Chloracne (193 cases in 1978), children primarily
  - TCDD levels in blood of 2,000-56,000 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, haemopoietic neoplasms, liver, breast cancer (slightly, not consistent)
  - Increased incidence Diabetes mellitus
  - Change of sex ratio (more girls)
  - Decreased sperm counts in boys exposed at infancy and by breast feeding
- Exposed group rather small for firm conclusions

Health risks dioxins overestimated ?

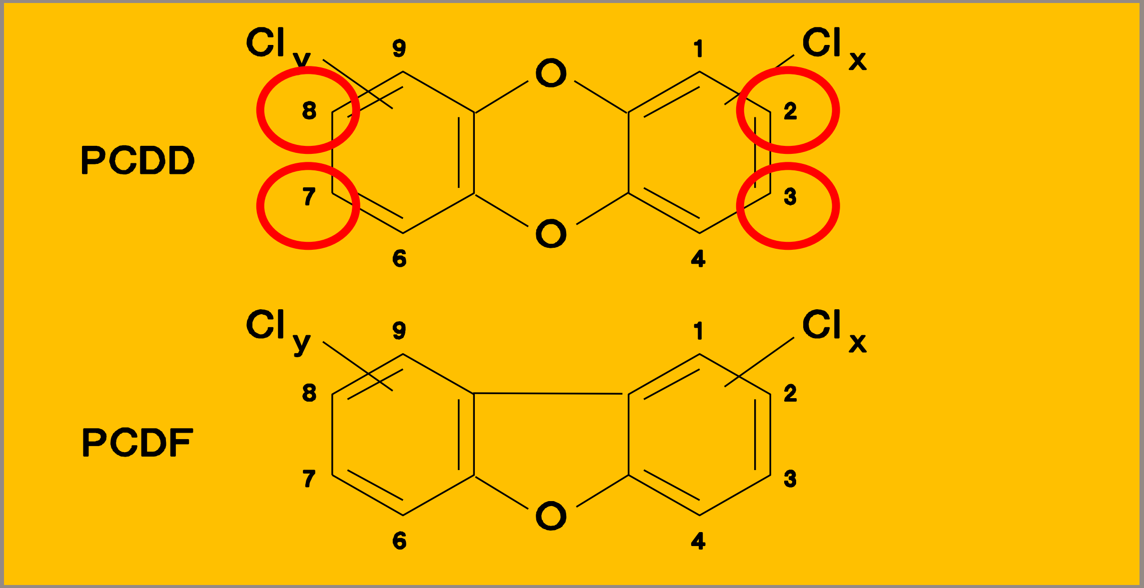
"It's the new symbol for chemicals the government says aren't so bad after all."

Dioxins and PCBs

- Which compounds ?
- Effects
- Risk assessment TCDD
- TEQ principle
- Incidents
- Prevention/impact reduction

Dioxins (PCDD/Fs)

polychlorinated dibenzo-p-dioxins and furans

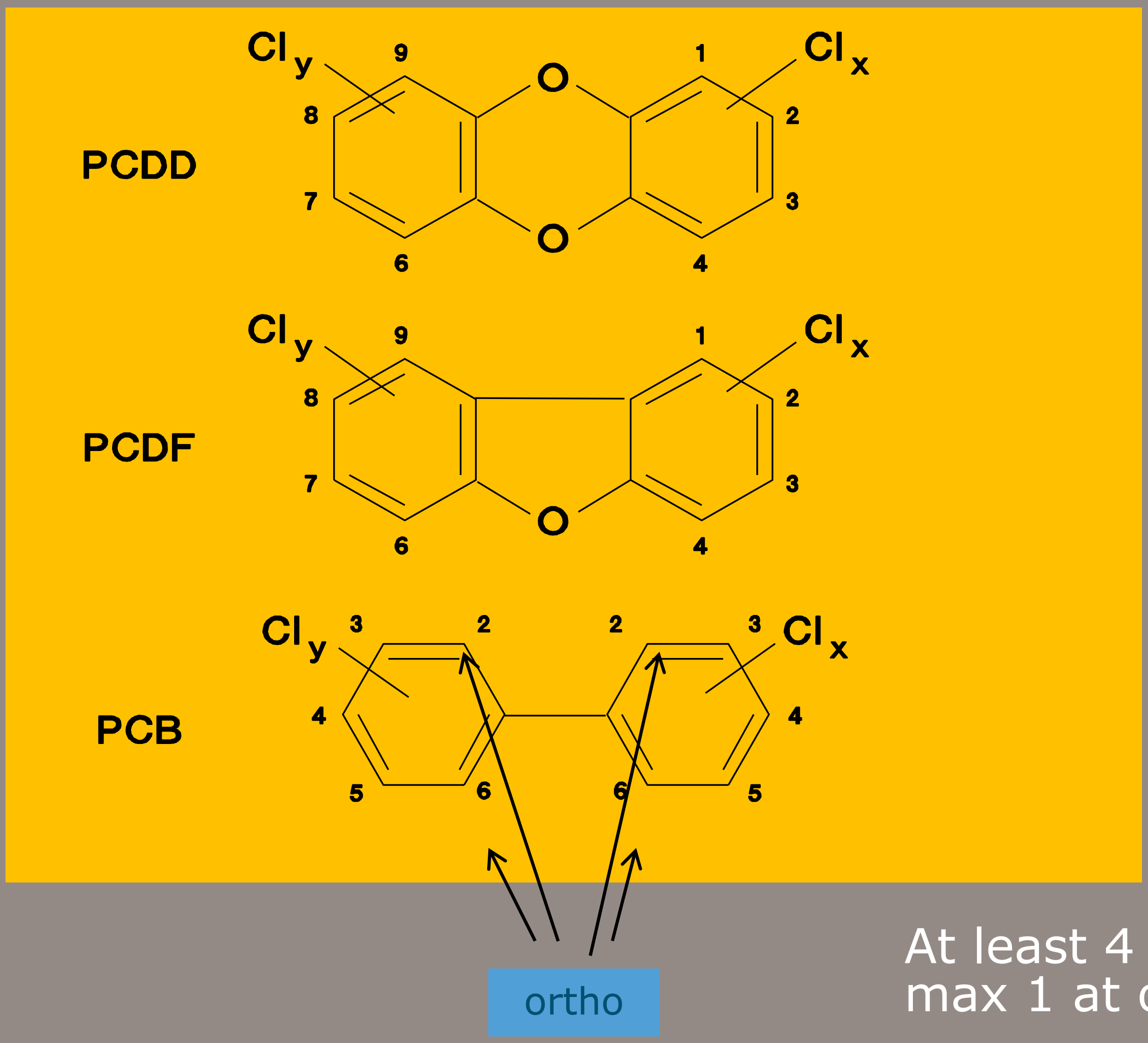


7 out of 75

10 out of 135

So at least 4 chlorines and positions 2,3,7 and 8 occupied

Dioxins (PCDD/Fs), and dioxin-like PCBs



7 out of 75

10 out of 135

12 out of 209

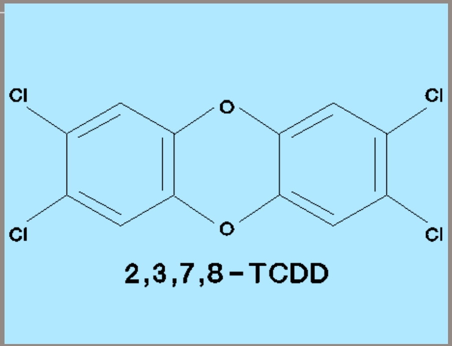
At least 4 chlorines and max 1 at ortho position

Properties of dioxins and planar PCBs

- Mixture of 29 congeners with different toxic potencies (including planar PCBs)
  - Most toxic congener: TCDD
- In test animals effects at very low doses
- Accumulation in fat
  - slow metabolism and elimination
  - But what is the critical level?

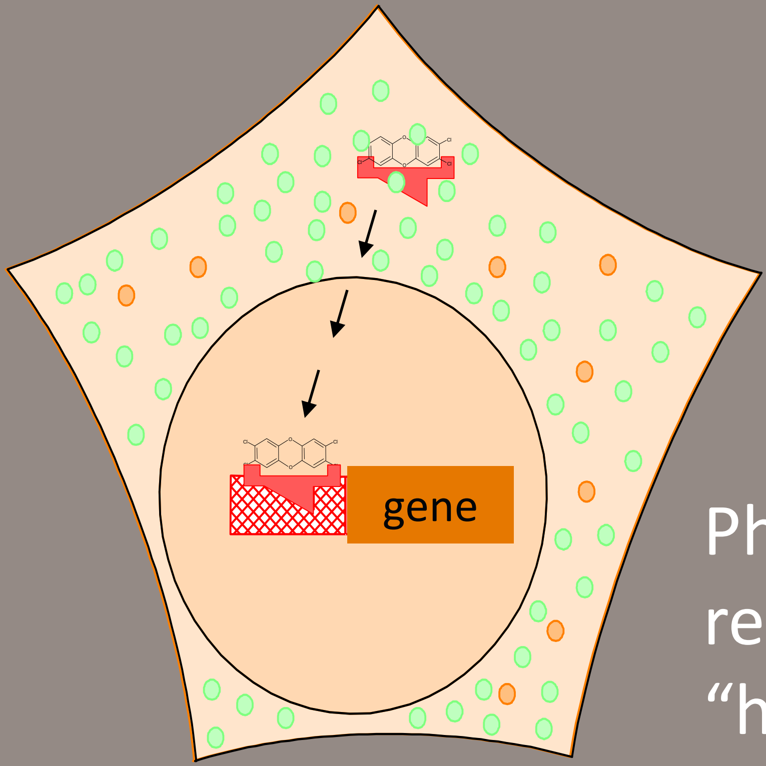
Toxicity of TCDD

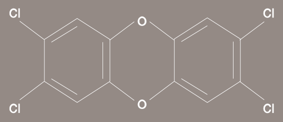
(2,3,7,8-tetrachlorodibenzo-p-dioxin)



- Most toxic congener
- Effects in laboratory animals
  - Endometriosis in monkeys
  - Neurobehavioral effects in monkeys
  - Immune suppression in offspring rats
  - Decreased sperm count in male offspring of rats
- Liver tumours in female rats at higher dose levels
  - Recognized human carcinogen (IARC)

Central role arylhydrocarbon receptor (AhR)





Physiological role Ah-receptor, and natural “hormone” unknown

Genes affected

- Enzymes involved in metabolism of endogenous compounds and xenobiotics
  - Cytochrome P450s 1A1, 1A2, 1B1
  - Some other cytochrome P450s
  - Aldehyde oxidase
  - Glucuronyltransferases
  - GSH transferases
- Some other genes
  - e.g. TIPARP

Mode of action?

Toxicity of TCDD: hazard characterization

Safe body burden concept

- Effects more related to actual levels in the body (body burden) than to intake levels
  - Actually to blood levels but in equilibrium with fat
  - At least for chronic exposure to relatively low levels
- Effects in animals at body burdens of 30-70 ng/kg b.w.

Studies used by WHO 1998

Study	Endpoint	Exposure (LOAEL)
Schantz and Bowman (1989)	Rhesus monkey, neurotoxicity (decreased learning)	~160 pg/kg b.w. per day
Gray et al. (1997); Mably et al. (1992)	Rat, decreased sperm count in offspring	64 ng/kg b.w.(c)
Gray et al. (1997)	Rat increased genital malformations in offspring	200 ng/kg b.w.(c)
Gehrs et al. (1997)	Rat immune suppression in offspring	100 ng/kg b.w.(c)
Rier et al. (1993)	Rhesus monkey, endometriosis	~160 pg/kg b.w./day

(a): Increment to background, reported to be 4 ng/kg (TEQ)., (b): Body burden at time of delivery, (c): Single oral dose, (d): Maternal body burden.

Studies used by WHO 1998

Study	Endpoint	Exposure (LOAEL)	Body burden[a] (ng/kg b.w.)
Schantz and Bowman (1989)	Rhesus monkey, neurotoxicity (decreased learning)	~160 pg/kg b.w. per day	42[b,d]
Gray et al. (1997); Mably et al. (1992)	Rat, decreased sperm count in offspring	64 ng/kg b.w.[c]	28[d]
Gray et al. (1997)	Rat increased genital malformations in offspring	200 ng/kg b.w.[c]	73
Gehrs et al. (1997)	Rat immune suppression in offspring	100 ng/kg b.w.[c]	50
Rier et al. (1993)	Rhesus monkey, endometriosis	~160 pg/kg b.w./day	69[b]

(a): Increment to background, reported to be 4 ng/kg (TEQ)., (b): Body burden at time of delivery, (c): Single oral dose, (d): Maternal body burden.

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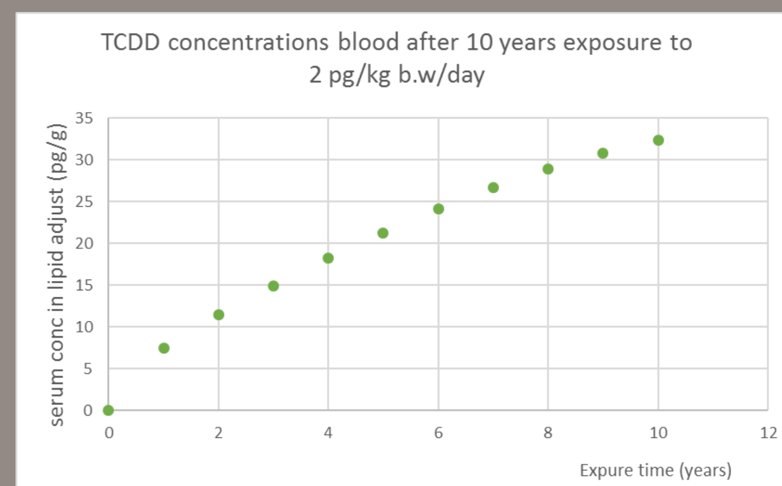
What is a safe body burden for humans?

- Factor of 3 to extrapolate LOAEL to NOAEL
- Normally use of factor of 10x10 for extrapolation of animals to humans
- Use of uncertainty factor of (only) 3.2 for possible inter-individual differences
  - Differences in kinetics accounted for when using body burden
  - Humans seem not more sensitive than rats
- So overall factor of 10 applied

Which intake results in safe body burden level?

In women of child bearing age

- How many drops per day to fill up the bucket?
- Or avoid that it over floats?



From BB to safe daily intake

- WHO: based on half-life in humans extrapolated to intake of 14-37 pg/kg b.w./day for humans

$$\text{Intake (ng/kg/day)} = \text{Body Burden (ng/kg)} \times (\ln(2)/\text{half-life})/f$$

  - f=absorption factor (50%)
  - Half-life = 7.5 yrs for humans

Studies used by WHO 1998

Study	Endpoint	Exposure (LOAEL)	Body burden[a] (ng/kg b.w.)	Related human EDI (pg/kg b.w. per day)
Schantz and Bowman (1989)	Rhesus monkey, neurotoxicity (decreased learning)	~160 pg/kg b.w. per day	42[b,d]	21
Gray et al. (1997); Mably et al. (1992)	Rat, decreased sperm count in offspring	64 ng/kg b.w.[c]	28[d]	14
Gray et al. (1997)	Rat increased genital malformations in offspring	200 ng/kg b.w.[c]	73	37
Gehrs et al. (1997)	Rat immune suppression in offspring	100 ng/kg b.w.[c]	50	25
Rier et al. (1993)	Rhesus monkey, endometriosis	~160 pg/kg b.w./day	69[b]	35

(a): Increment to background, reported to be 4 ng/kg (TEQ)., (b): Body burden at time of delivery, (c): Single oral dose, (d): Maternal body burden.

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Tolerable daily intake (TDI)

- TDI of 1-4 pg TEQ/kg b.w./day (WHO)
- Scientifically sensible to give a range
- But how to use it as risk manager?

SCF 2000, with update in 2001

- Most sensitive study Faqi et al. 1998
  - Based on sperm effects in rats, exposed in utero
  - LOAEL BB 40 ng/kg bw
  - Including correction factor for peak exposure
- EHDI of 20 pg TEQ/kg bw/day
  - Using uncertainty factor of 10 (3x3.2)
  - TDI of 2 pg TEQ/kg bw/day
- TWI of 14 pg TEQ/kg b.w./week
  - Aiming at prevention of high body burden mother

Decreased sperm production in male offspring rats (Faqi et al. 1998)

TABLE 3

Effect of *In Utero* and Lactational TCDD Exposure on Sperm Number/Cauda Epididymis, Daily Sperm Production, Sperm Transit Rate, Sperm Morphology, and Serum Testosterone Concentration Investigated at Days 70 and 170 Postnatally

Parameters	Control	TCDD 25/5	TCDD 60/12	TCDD 300/60
PND 70				
Number of animals	20	20	20	20
Sperm number from cauda epididymis ($\times 10^6$)	209 ± 43	176 ± 38*	203 ± 42	172 ± 52*
Daily sperm production ($\times 10^6$)	34.4 ± 4.3	28 ± 5.7*	25.2 ± 5.6*	23.1 ± 4.9*
Sperm transit rate (days)	6.1 ± 1.5	6.5 ± 2.0	8.4 ± 2.7**	7.8 ± 3.0**
Testosterone concentration (ng/ml)	2.08 ± 1.1	2.1 ± 1.0	2.92 ± 1.6	2.7 ± 1.5
PND 170				
Number of animals	20	20	20	20
Sperm number from cauda epididymis ($\times 10^6$)	326 ± 75	270 ± 61*	235 ± 44*	257 ± 86*
Daily sperm production ($\times 10^6$)	45.6 ± 6.2	27.5 ± 7.2*	24.8 ± 5.9*	23.4 ± 5.6*
Sperm transit rate (days)	7.4 ± 2.3	10.6 ± 4.0**	10.0 ± 3.6**	12.1 ± 4.3**
Percent of abnormal sperm	7.3 ± 2.1	10.9 ± 3.3*	14.1 ± 3.5*	12.4 ± 4.2*
Testosterone concentration (ng/ml)	2.2 ± 1.1	2.3 ± 1.6	1.7 ± 1.1	1.2 ± 0.7*

Note. Values are means ± SD. *Values are significantly decreased from control values. **Values are significantly increased from control values.

Single dose of 25, 60 or 300 and weekly dose of 5, 12 or 60 ng/kg bw

JECFA (2001)

- JECFA: TMI of 70 pg TEQ/kg b.w./month
  - Same studies used
- Further extension possible, e.g. TYI?
  - Not necessarily,
  - single high dose (incident!) may give increased blood level, and thus
  - higher exposure of e.g. foetus
  - So depends on effect intake on blood levels
- So: exposure is chronic, but effects may be (more) acute

Exposure limit US-EPA (Reference dose)

- US-EPA (2012): RfD of 0.7 pg TEQ/kg bw/day
- Based on new human studies from Seveso
  - Effects on sperm production in men exposed as young boys (Mocarelli et al. 2008)
  - Effects on thyroid hormones in children exposed in utero (Baccarelli et al. 2008)
- Use of human PBK-model (Emonds)
  - Physiologically based kinetics
- Uncertainty factor of 30 applied (10 for LOAEL/NOAEL extrapolation)

Mocarelli et al. 2008

Characteristic by age group	EG 22–31 (1–9)	EG 32–39 (10–17)	CG 22–31	CG 32–39	p- EG vs. CG 22–31	p- EG vs. CG 32–39
Participants (n)	71	44	82	71		
TCDD exposure (ppt[b] level median)						
In 1976	210[c]	164[c]	≤ 15[d]	≤ 15[d]		
In 1998	3.04	4.67	< 6.0	< 6.0		
Sperm concentration (10^6/mL)					0.025	0.213
Mean[e]	53.6	81.9	72.5	60.8		
Mean ± SD[e]	21.8–131.8	37.8–177.9	31.7–165.9	24.2–152.8		
Adjusted mean[e]	48.6	87.4	67.1	70.5		
Adjusted mean ± SE	43.1–54.8	74.7–102.3	59.4–75.7	61.3–81.1		

Effects in man exposed at young age (1-9 years)

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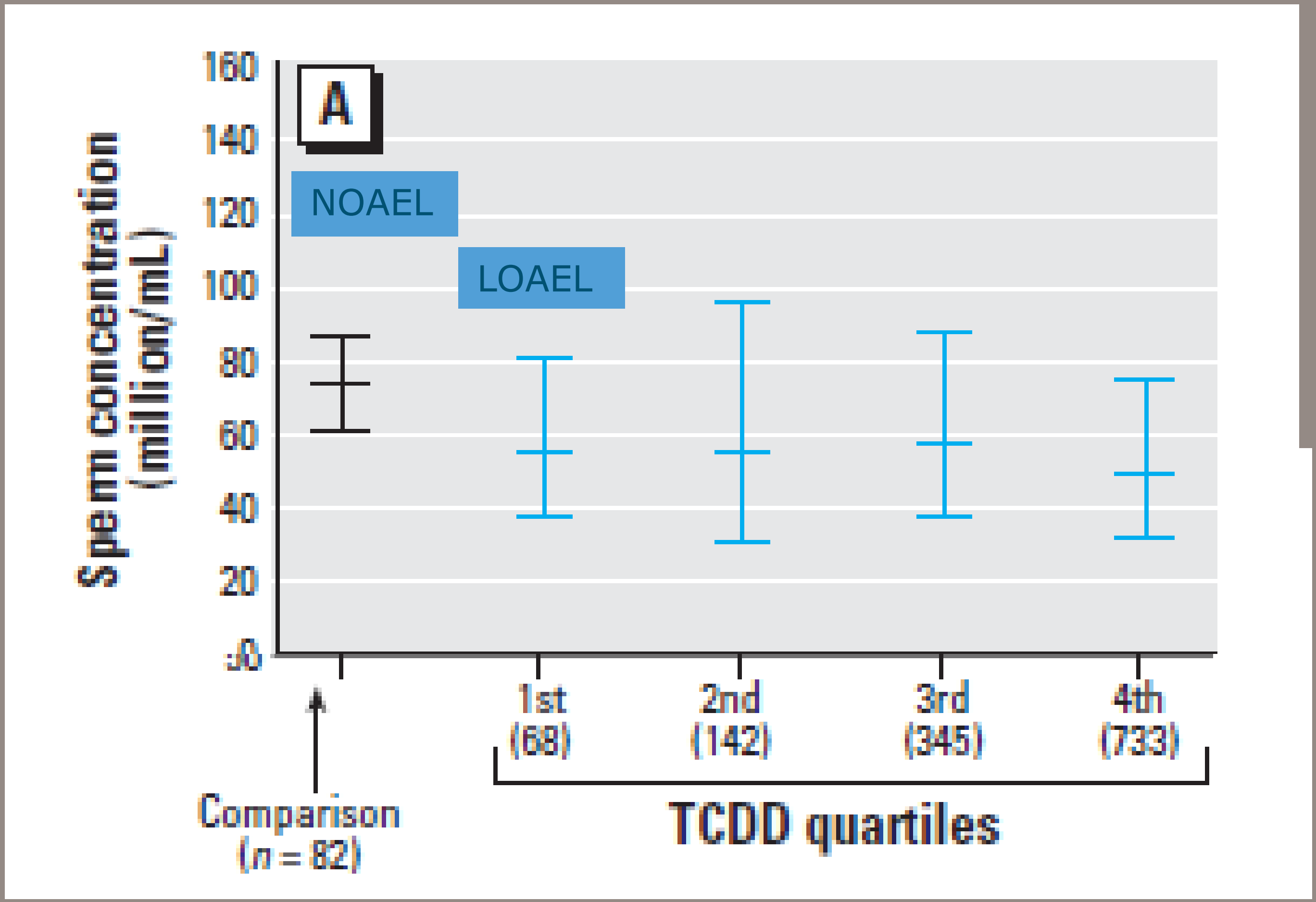
Dose-response? (split-up young boys)



No clear dose-response: LOAEL may be much lower than 68 ppt)

Mocarelli et al. 2008

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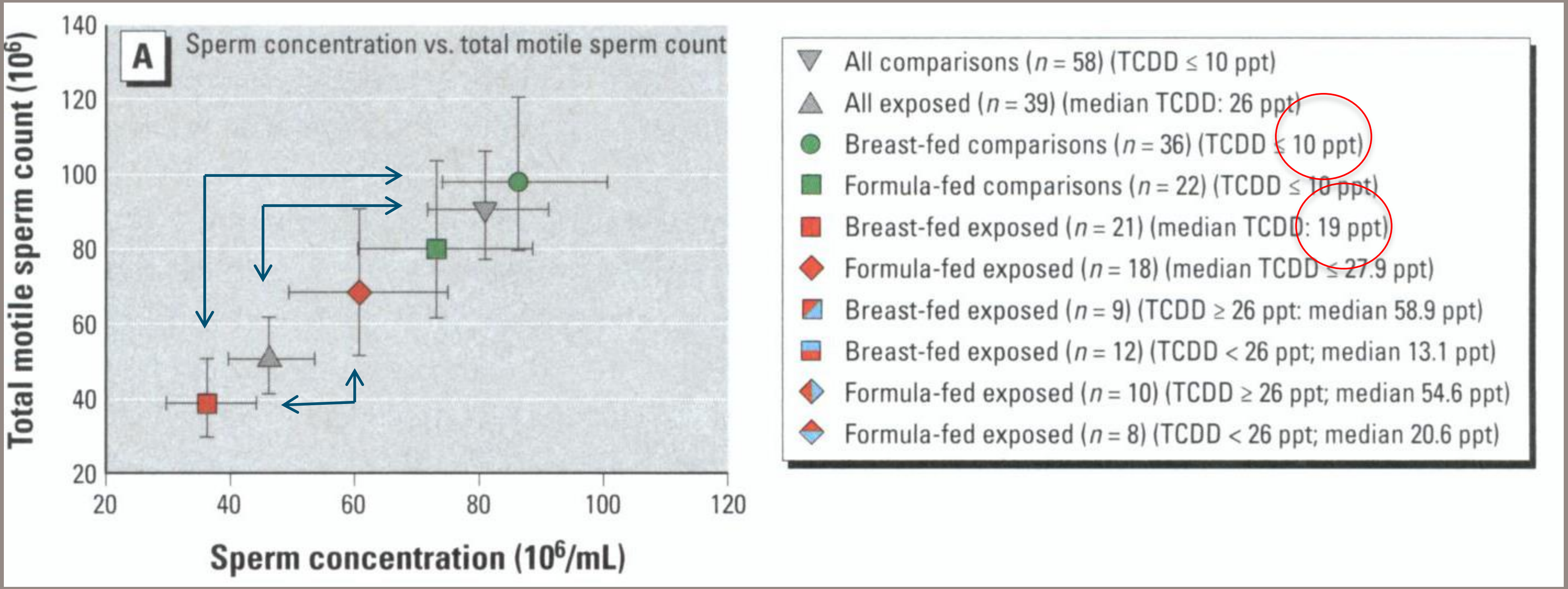
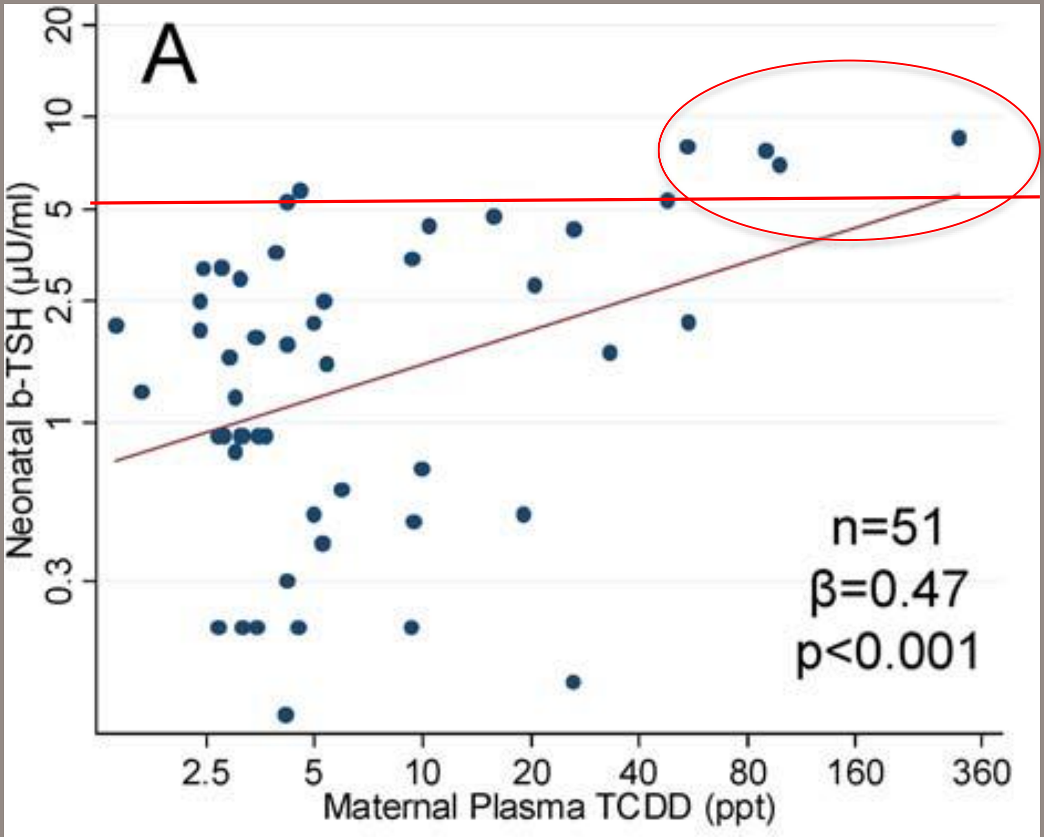
Mocarelli et al. 2011: perinatal exposure



- Clear effect on breast fed children (effects at lower BB)
- Not yet used for risk assessments

Baccarelli et al. 2008: TSH levels



Increased TSH levels in babies of exposed mothers: LOAEL of 235 ppt (based on levels>5: possible hypothyroidy)

Derivation RfD

- Using blood levels of 68 (boys) and 235 (mothers) pg/g fat as point of departure
- Use PBK model to estimate daily dose leading to those body burdens: 20 pg TCDD/kg bw/day for both
- Applying uncertainty factors
  - factor 10 to derive NOAEL from LOAEL
  - factor 3 for intraspecies differences
- RfD of 0.7 pg TEQ/kg bw/day
  - So 3x lower than TWI (SCF) and TMI (JECFA)
- Current exposure in range RfD-TWI
- EFSA asked for new risk assessment

New data to take into account?

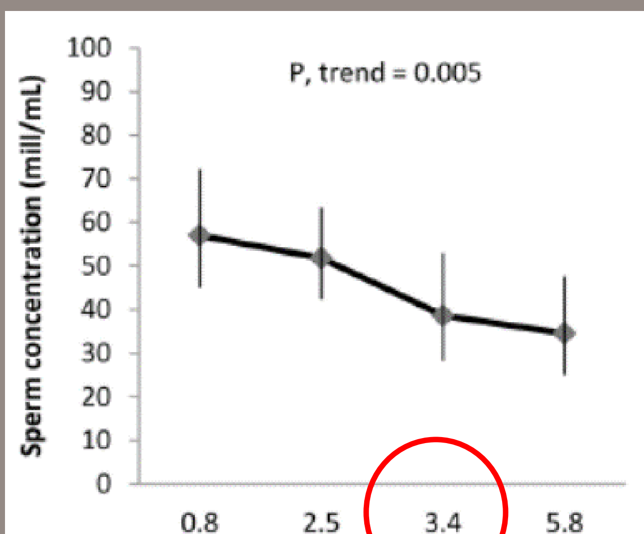
- Seveso study breast feeding
- Russian children study in Chapaevsk, Russia
    - Former production chlorinated pesticides
    - Blood levels boys taken at 8-9 yrs (2003-2005)
        - Chlorinated pesticides, lead
        - PCDD/Fs and DL-PCBs
    - Delayed onset of puberty
    - Effects on sperm counts (18-19 yrs; n=133)

Mocarelli et al., 2011, Minguez-Alarcon et al., 2017

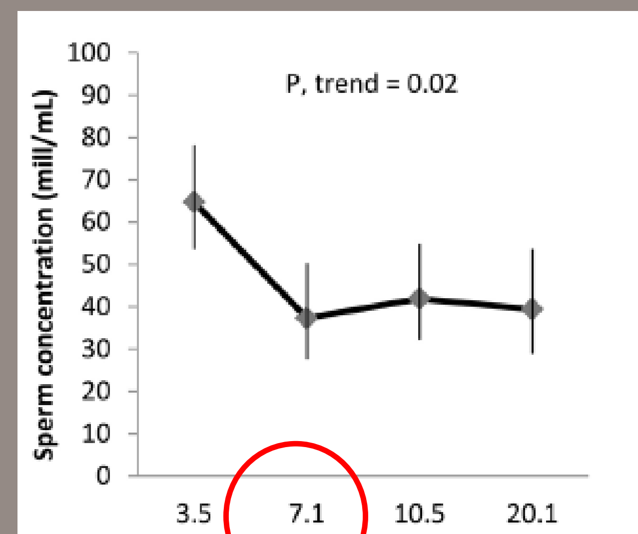
Association TCDD and sperm concentration

Table 3. Multivariable adjusted mean semen parameters by quartiles (Q)[a] of serum dioxins, furans, and PCBs among 133 young men in the Russian Children's Study contributing 256 semen samples.

Toxic equivalent/ concentration	Volume (mL)	Sperm concentration (million/mL)	Total sperm count (million)	Motile sperm (%)	Total motile sperm count (million)
TEQs (pg TEQ/g lipid)					
TCDD					
Q1 (0.35–1.70)	2.7 (2.2, 3.2)	57.0 (45.0, 72.1)	128 (95.6, 173)	61.6 (58.6, 64.7)	78.0 (56.0, 109)
Q2 (1.77–2.45)	2.9 (2.5, 3.4)	51.8 (42.4, 63.3)	136 (105.0, 175)	65.4 (63.4, 67.4)	87.9 (67.1, 115)
Q3 (3.00–3.40)	2.6 (2.1, 2.9)	38.6 (28.2, 52.9)*	85.8 (60.4, 122)	59.5 (56.0, 62.9)	50.1 (33.5, 74.8)
Q4 (4.40–5.80)	3.1 (2.5, 3.7)	34.5 (25.0, 47.7)*	91.6 (63.5, 132)	60.1 (56.6, 63.7)	54.1 (36.0, 81.4)
p-trend	0.55	0.005	0.05	0.17	0.05



Also other PCDD/Fs and DL-PCBs



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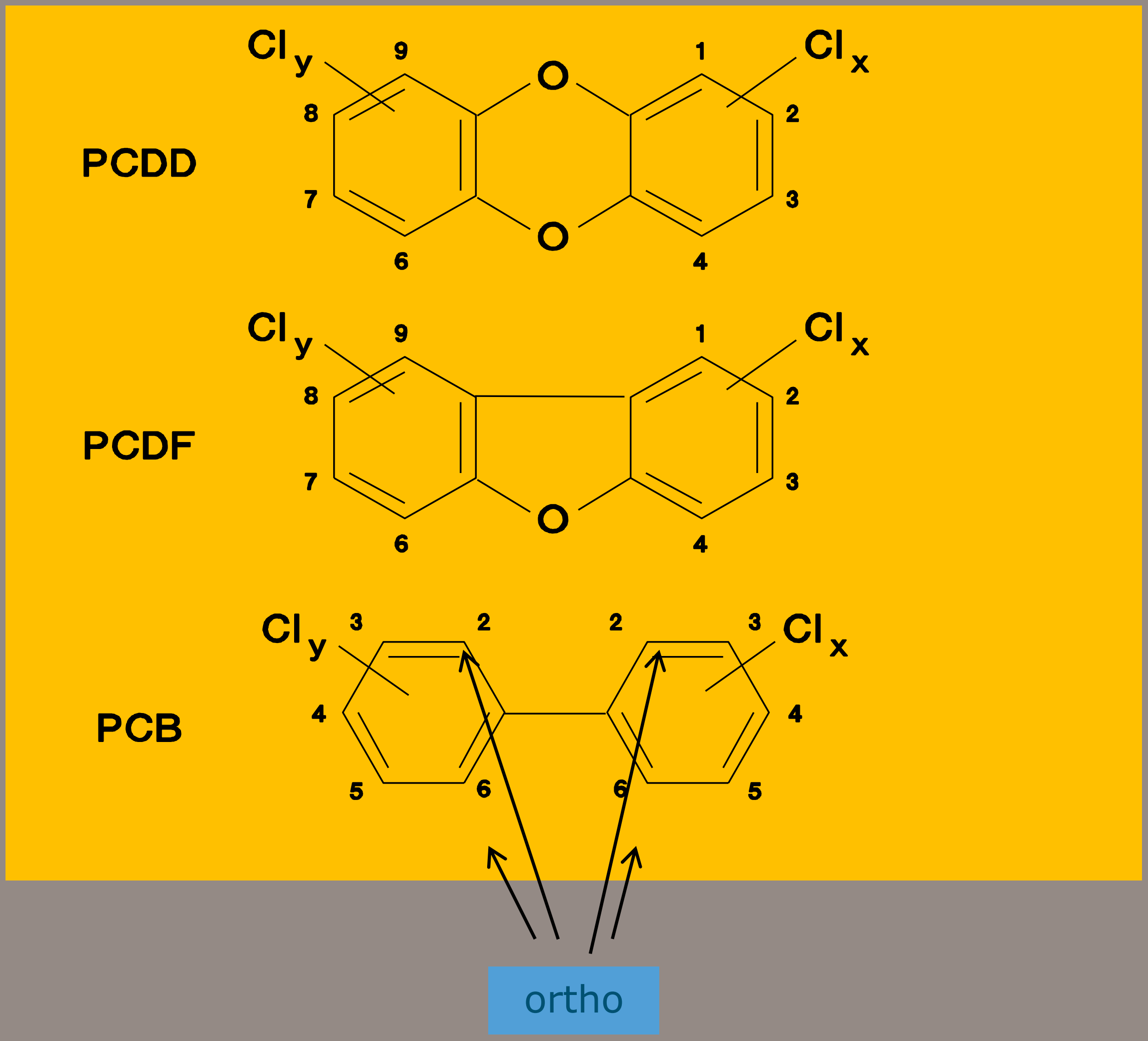
Mixtures of dioxins and DL-PCBs

Dealing with mixtures



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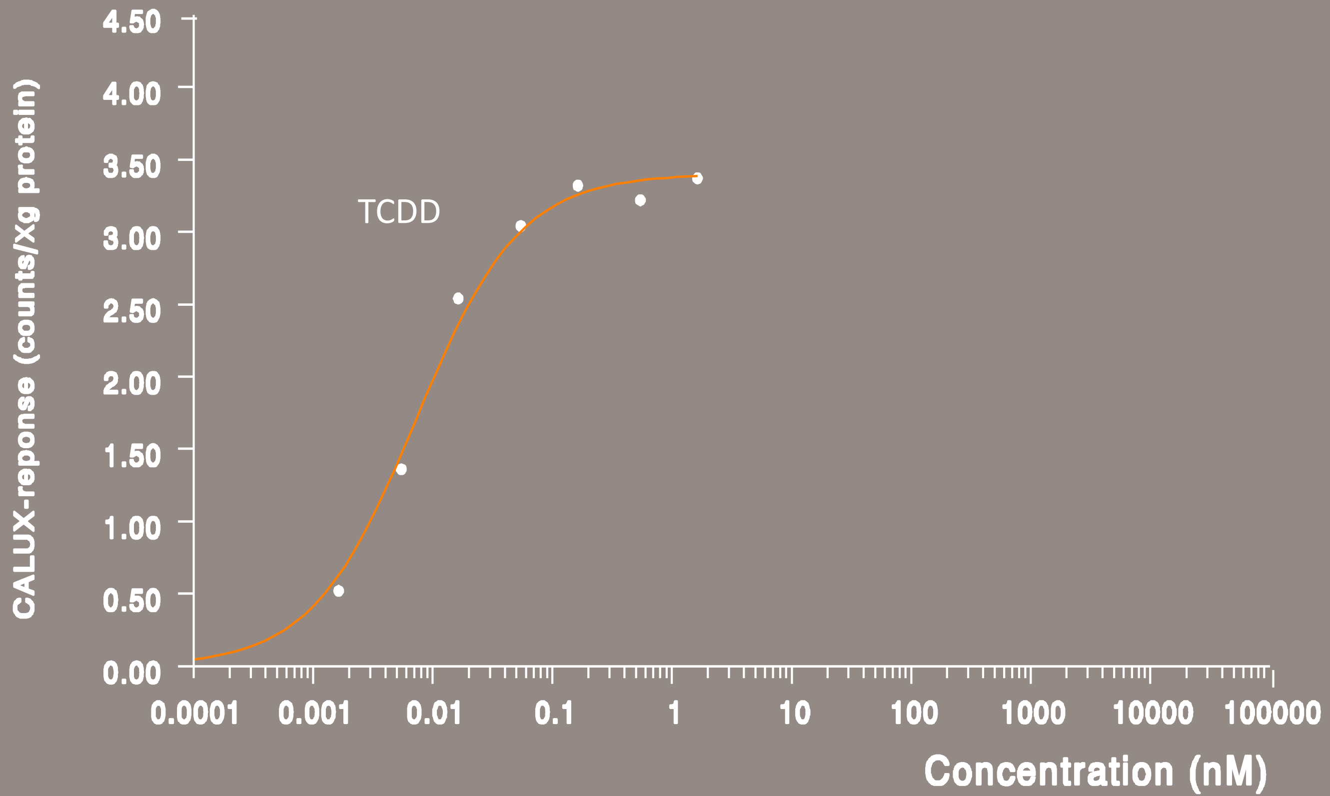
10 out of 135

12 out of 209

Dose-response curves for dioxins and dl-PCBs



Bovee et al. 1998

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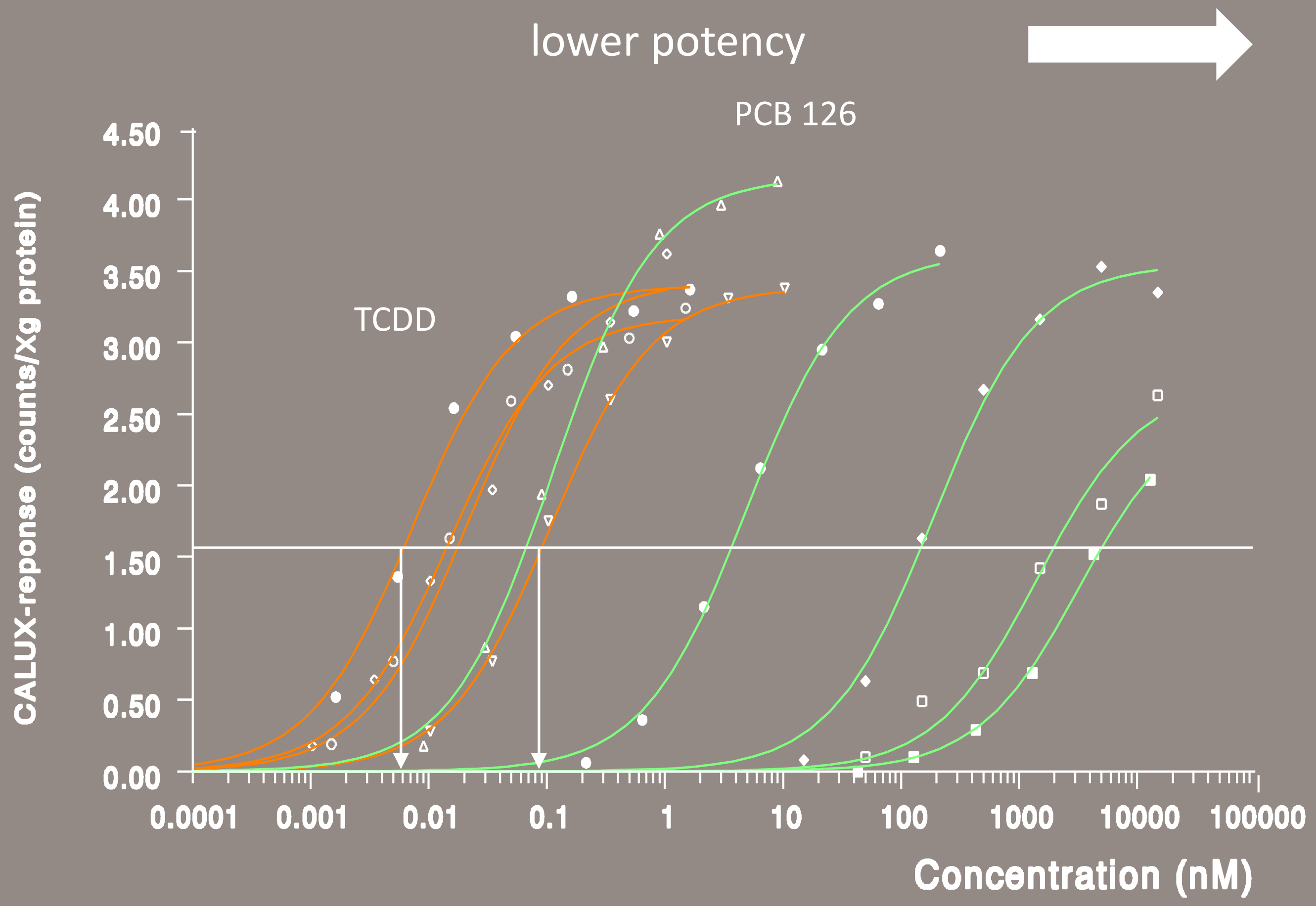
Dose-response curves for dioxins and dl-PCBs



Bovee et al. 1998

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Risk assessments for TCDD

- Health based guidance values (TWI, TMI, RfD) apply for sum of dioxins and dioxin-like PCBs
- How to deal with a mixture of congeners with different toxic potencies?

TEF values (Toxic Equivalency Factors)

TEQ-principle:

- Estimate the toxic potency of every dioxin and dioxin-like PCB in comparison to TCDD
- Various schemes proposed:
    - I-TEFs, Nordic TEFs for DL-PCBs, WHO_{1998}, WHO_{2005}
- Based on in vivo and in vitro studies (relative potency factors or REPs): range of values for each congener
- Each dioxin and PCB obtains a TEF value
    - TEF for TCDD: 1
    - Current range: 1-0.00003

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
- Regularly evaluated (last time 2005)

Change in TEF-values in 2005

congener	WHO TEF (1998)	WHO TEF (2005)
2,3,7,8-TCDF	0.1	0.1
2,3,7,8-TCDD	1	1
1,2,3,7,8-PeCDF	0.05	0.03
2,3,4,7,8-PeCDF	0.5	0.3
1,2,3,7,8-PeCDD	1	1
1,2,3,4,7,8-HxCDF	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1
1,2,3,4,7,8-HxCDD	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.01	0.01
1,2,3,4,6,7,8-HpCDD	0.01	0.01
OCDF	0.0001	0.0003
OCDD	0.0001	0.0003

Change in TEF-values

congener	WHO TEF (1998)	WHO TEF (2005)
Non-ortho PCBs		
PCB 81	0,001	0,001
PCB 77	0.001	0.003
PCB 126	0.1	0.1
PCB 169	0.01	0.03
Mono-ortho PCBs		
PCB 105	0.0001	0.00003
PCB 114	0.0005	0.00003
PCB 118	0.0001	0.00003
PCB 123	0.0001	0.00003
PCB 156	0.0005	0.00003
PCB 157	0.0005	0.00003
PCB 167	0.00001	0.00003
PCB 189	0.0001	0.00003

Application TEFs

Level of mixture expressed in TEQ:

- TEQ = Σ (congener$_i$ level) x TEF$_i$

Calculation TEQ level: Belgian feed 1999

congener	WHO TEF (1998)	Level ng/kg	ng TEQ/kg
2,3,7,8-TCDF	0,1	363	36
2,3,7,8-TCDD	1	23	23
1,2,3,7,8-PeCDF	0,05	274	14
2,3,4,7,8-PeCDF	0,5	1136	568
1,2,3,7,8-PeCDD	1	59	59
1,2,3,4,7,8-HxCDF	0,1	473	47
1,2,3,6,7,8-HxCDF	0,1	78	8
2,3,4,6,7,8-HxCDF	0,1	175	18
1,2,3,7,8,9-HxCDF	0,1	23	2
1,2,3,4,7,8-HxCDD	0,1	42	4
1,2,3,6,7,8-HxCDD	0,1	0	0
1,2,3,7,8,9-HxCDD	0,1	9	1
1,2,3,4,6,7,8-HpCDF	0,01	163	2
1,2,3,4,7,8,9-HpCDF	0,01	0	0
1,2,3,4,6,7,8-HpCDD	0,01	11	0
OCDF	0,0001	41	0
OCDD	0,0001	13	0
		2883	782

Calculation TEQ level: Belgian feed 1999

congener	WHO TEF (2005)	Level ng/kg	ng TEQ/kg
2,3,7,8-TCDF	0,1	363	36
2,3,7,8-TCDD	1	23	23
1,2,3,7,8-PeCDF	0,03	274	8
2,3,4,7,8-PeCDF	0,3	1136	341
1,2,3,7,8-PeCDD	1	59	59
1,2,3,4,7,8-HxCDF	0,1	473	47
1,2,3,6,7,8-HxCDF	0,1	78	8
2,3,4,6,7,8-HxCDF	0,1	175	18
1,2,3,7,8,9-HxCDF	0,1	23	2
1,2,3,4,7,8-HxCDD	0,1	42	4
1,2,3,6,7,8-HxCDD	0,1	0	0
1,2,3,7,8,9-HxCDD	0,1	9	1
1,2,3,4,6,7,8-HpCDF	0,01	163	2
1,2,3,4,7,8,9-HpCDF	0,01	0	0
1,2,3,4,6,7,8-HpCDD	0,01	11	0
OCDF	0,0003	41	0
OCDD	0,0003	13	0
		2883	548 (70%)

Establishment TEFs

- Change of TEFs has effect on TEQ levels
    - Last change: about 15% reduction in levels
- Last revised in 2005, but only since 2012 applied for official control in EU: check against product limits

New change in TEFs required?

- Are mono-ortho PCBs really AhR agonists?
  - But TEFs already low
  - So contribution is low, even with higher levels
- What about PCB 126 (most potent dl-PCB)?
  - In human cells relative low potency compared to TCDD
  - Based on enzyme induction, not adverse effects
  - Reduction would have huge impact on TEQ levels
  - Data enough to reduce the TEF? How to study in humans?

Prevention and impact reduction

Incidents in the food chain (lessons learned)

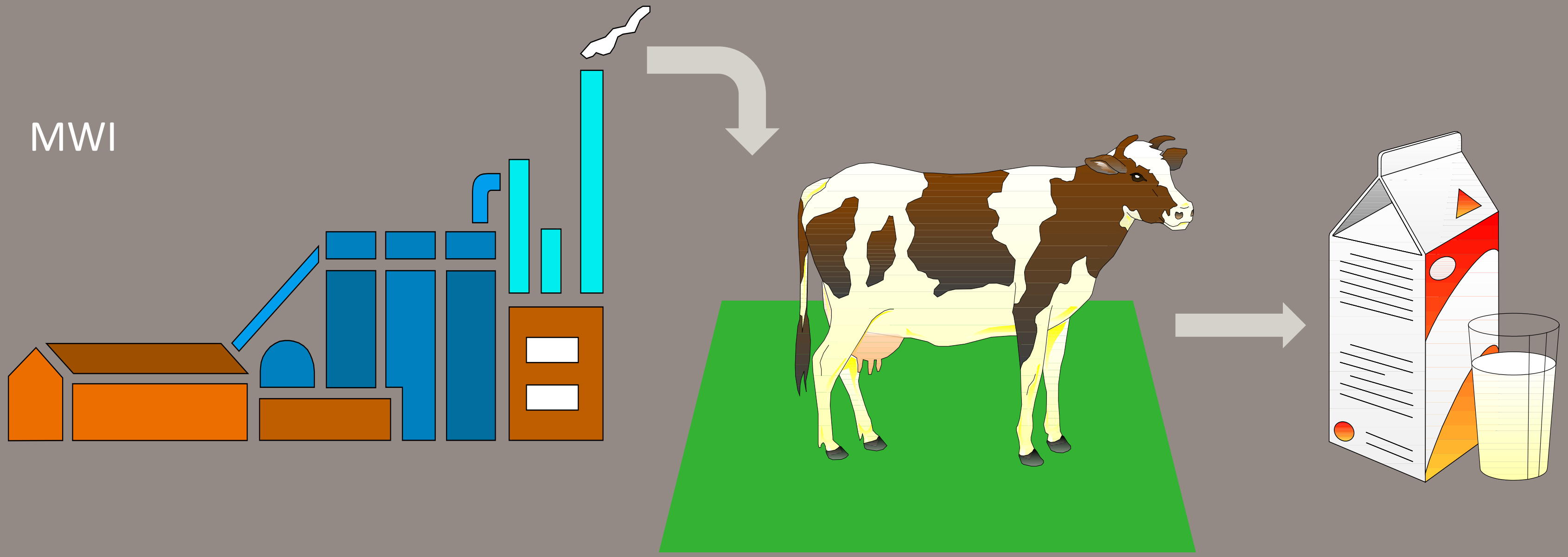
1957: chickens discover dioxins

- 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

Oil disease: YuSho (1967), YuCheng (1979)

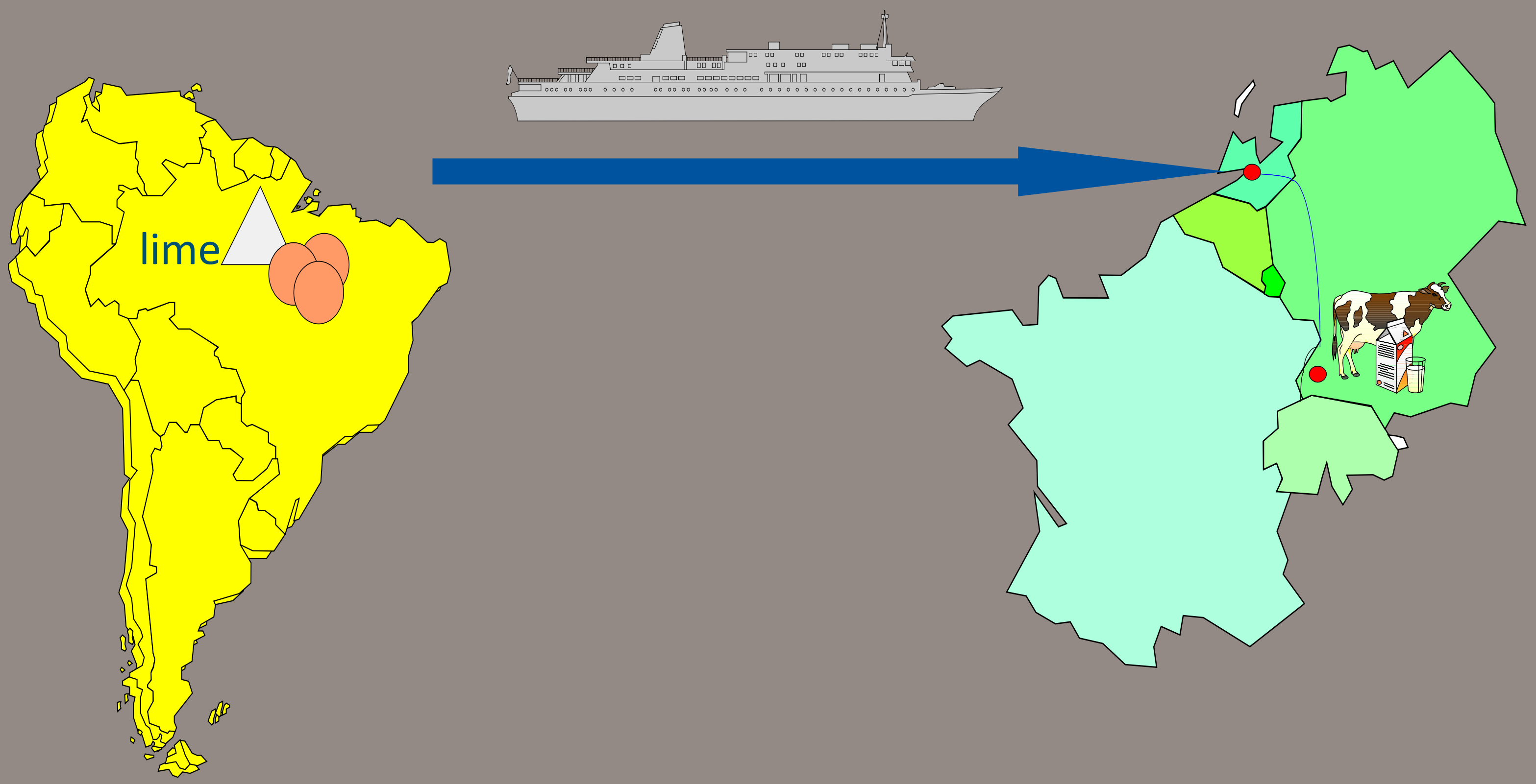
- Contamination of rice oil with PCB-oil, used as heat transfer fluid
  - Yusho (Japan) 2000 people exposed
  - Yucheng (Taiwan) 2000 people exposed
    - Used for 9 months
    - Average exposure 1 g PCBs, 4 mg PCDFs
    - TEQ levels around 40 ng TEQ/g fat
- Many people with chloracne
- Also chickens affected (fatty acid distillate)

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)



Use of contaminated lime for lowering water content and pH increase

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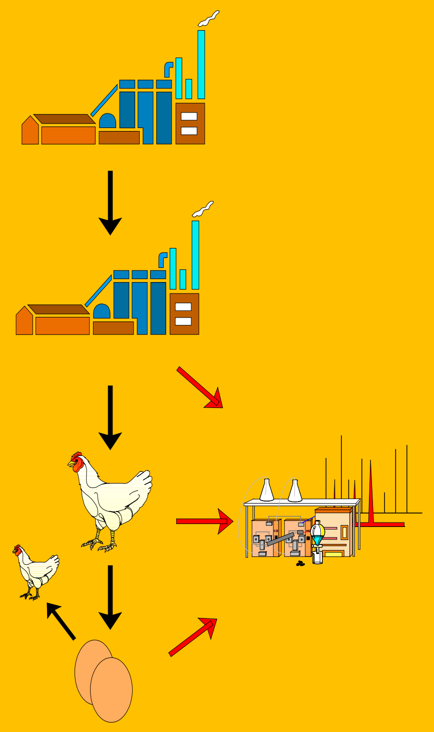
The Belgian dioxin crisis in 1999

Dioxins again discovered by chickens

- Decreased hatching
- No deficiency



Dioxins & PCBs in feed, chicken and eggs

Sample	Dioxins* (pg WHO-TEQ/g)	no-PCBs** (pg WHO-TEQ/g)	ind-PCBs*** (µg/g)
Animal feed	782	361	32
Chicken fat	958	453	37
Egg fat	685	ND	35

*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: 160 liter PCB-oil !

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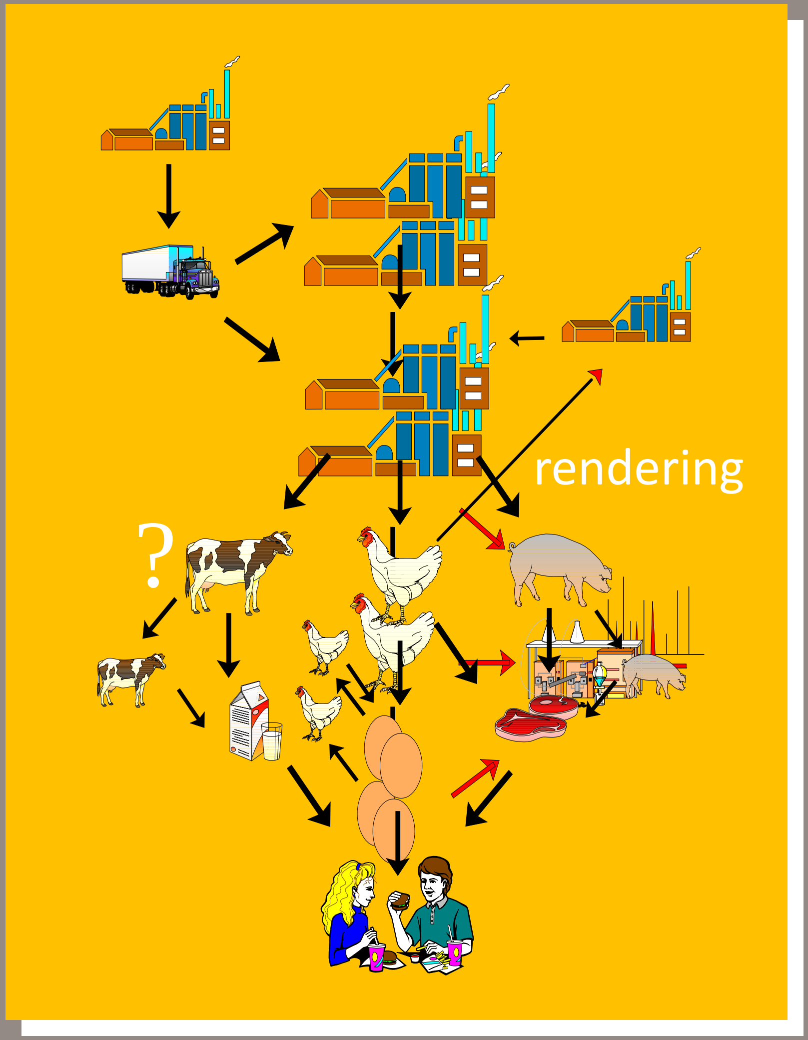
Development of the crisis



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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?

Food and feed incidents (not exclusive)

MWI milk 1989

Brazilian citrus pulp 1998

Belgian PCB fat 1999

German kaolinic clay 1999

Mozzarella Italy 2001-2004

Belgian choline chloride 2002

German bakery waste 2003

Potato peels/kaolinic clay 2004

Gelatin fat/Hydrochloric acid 2006

Indian Guar Gum 2007

Minerals (Zinc) Chile 2008

Bakery waste Ireland 2008

Organic corn Ukraine 2010

Fatty acids Germany 2011

Beet pulp Germany 2011

Free range eggs

Dioxins in Irish meat (december 2008)

From The Sunday Times
December 13, 2008

Pig feed toxins 'were off the scale'

The levels of dioxins found in Ireland's pig meat

Irish incident

- Discovered in France
  - Meat imported from the Netherlands
  - Traced back to Ireland
- Due to contaminated bakery waste
  - Dried on fire from oil containing PCBs
- Levels the highest ever seen in pork and beef
  - Up to 600 pg TEQ/g fat in pork
  - Even higher in cows (but fewer contaminated)
- Ongoing for at least 3 months

How to detect dioxins and/or DL-PCBs?

Methods?

GC/HRMS or GC/MS/MS: reference method

- GC/HRMS: confirmation
  - detection at pg/g levels
  - removal of fat
  - removal of pesticides
  - removal non-dl PCBs
  - detection with GC/HRMS or GC/MS/MS
- Different columns needed
  - Automated clean-up
  - Use of 13C-standards
  - Expensive method

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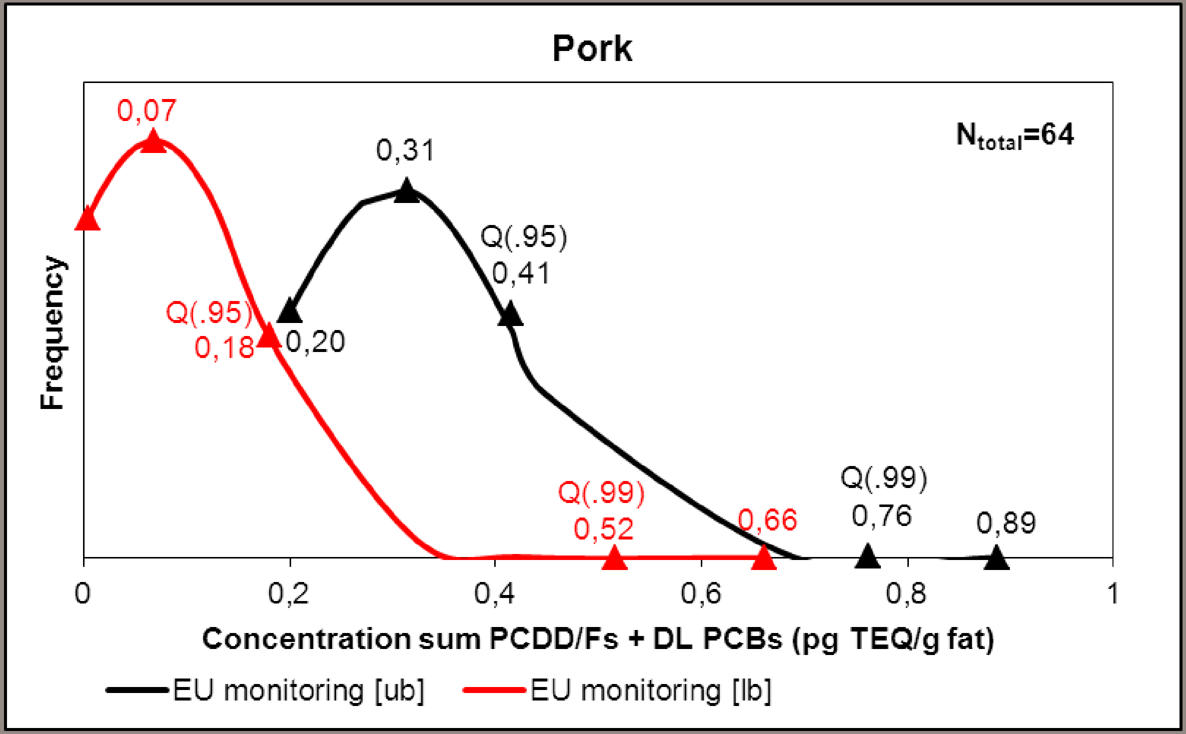
Relevant issues confirmatory methods

- Application of lower- and upperbound principle
    - Upperbound: levels of non-detected congeners are assumed to be equal to zero/LOQ
    - Lowerbound: non-detects set to zero
- Upperbound level used for checking compliance
- And for exposure assessment

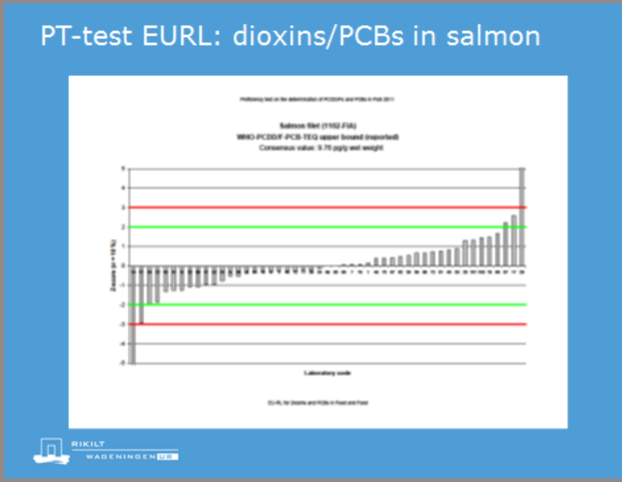
Effect ub vs lb for samples with low levels



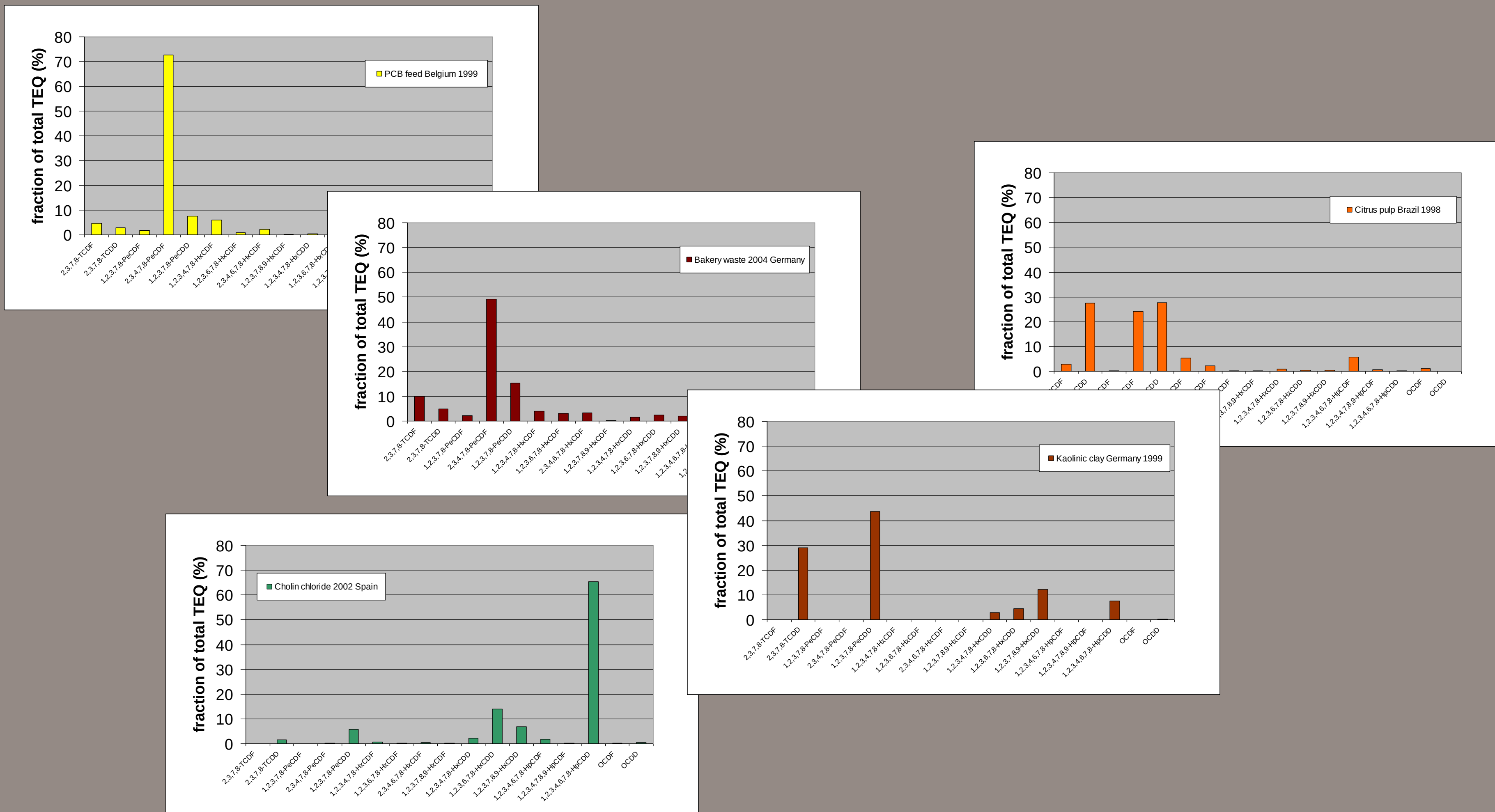
- Upperbound levels are a clear overestimation of the level
- So GC/HRMS levels not necessarily the truth

Relevant issues confirmatory methods

- Measurement uncertainty
  - The measurement uncertainty of the method should be established and levels corrected for this
  - Based on reproducibility of the analysis
  - So e.g. 1.15 may be reduced to 1.0 before checking compliance (15% MU)
- Also take into account result PT test (bias)
  - E.g. due to less good standards



GC/HRMS: allows use of patterns



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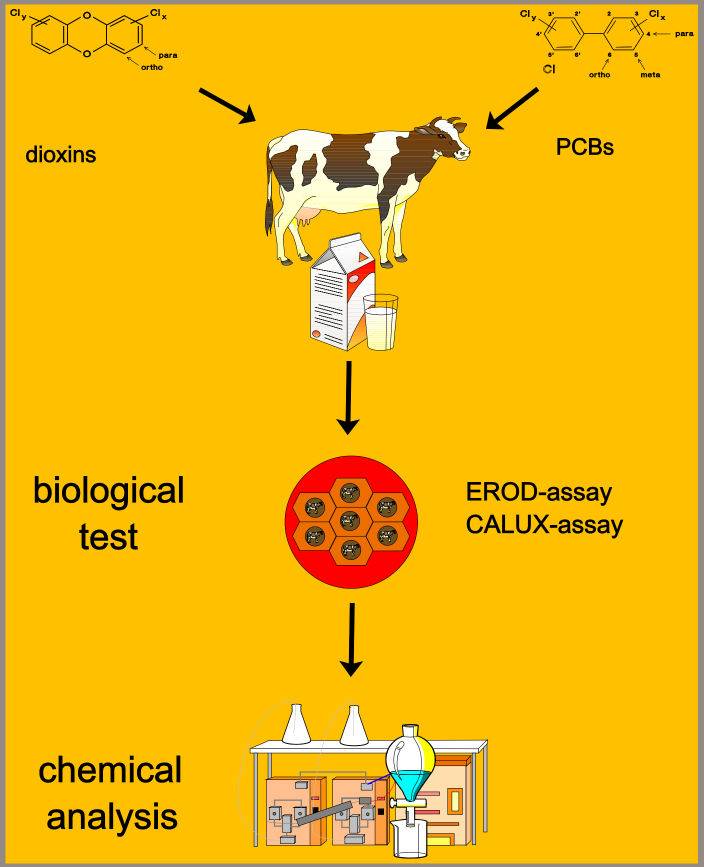
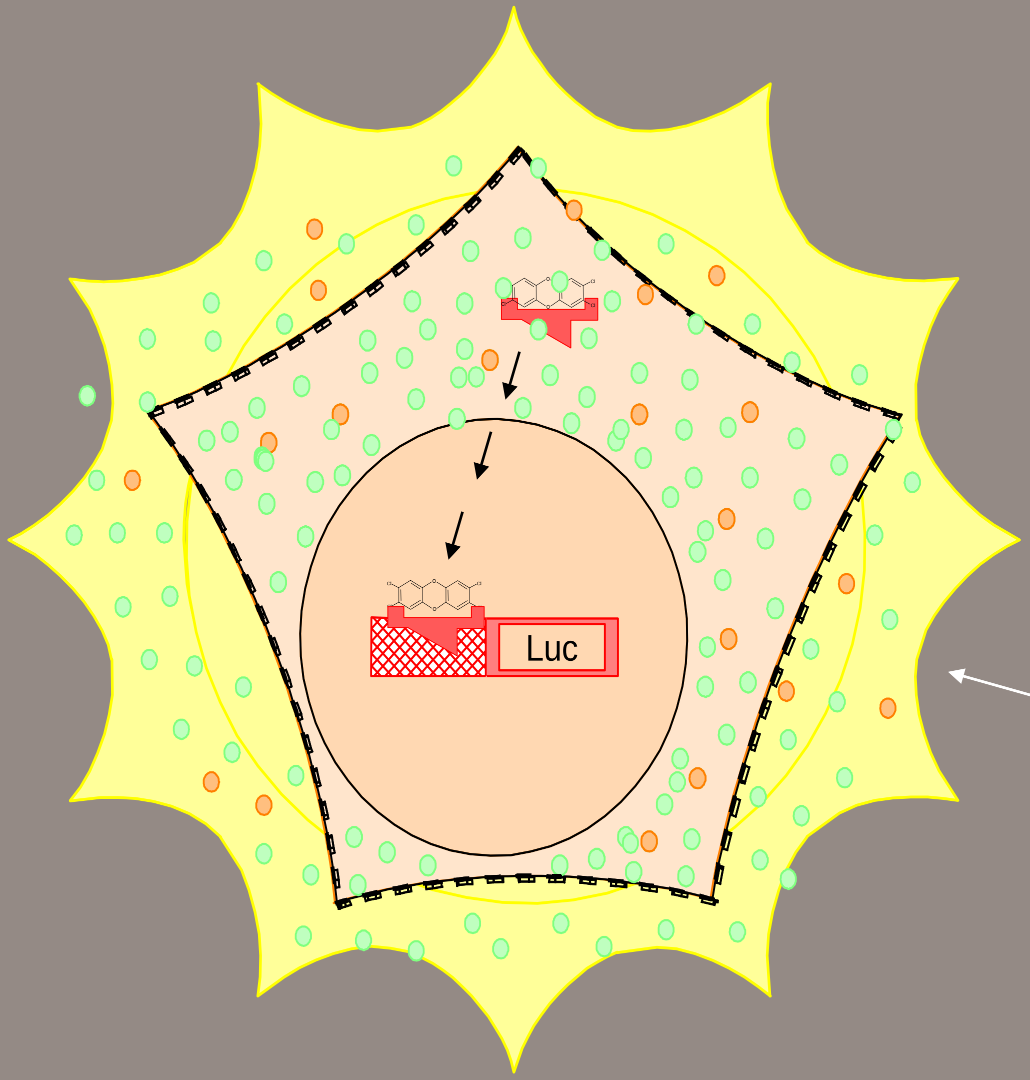
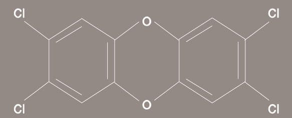
CALUX bioassay

- (DR) CALUX: screening
  - removal negative samples
  - confirmation suspects
- At RIKILT used since 1998
  - Almost every week



CALUX screening assay

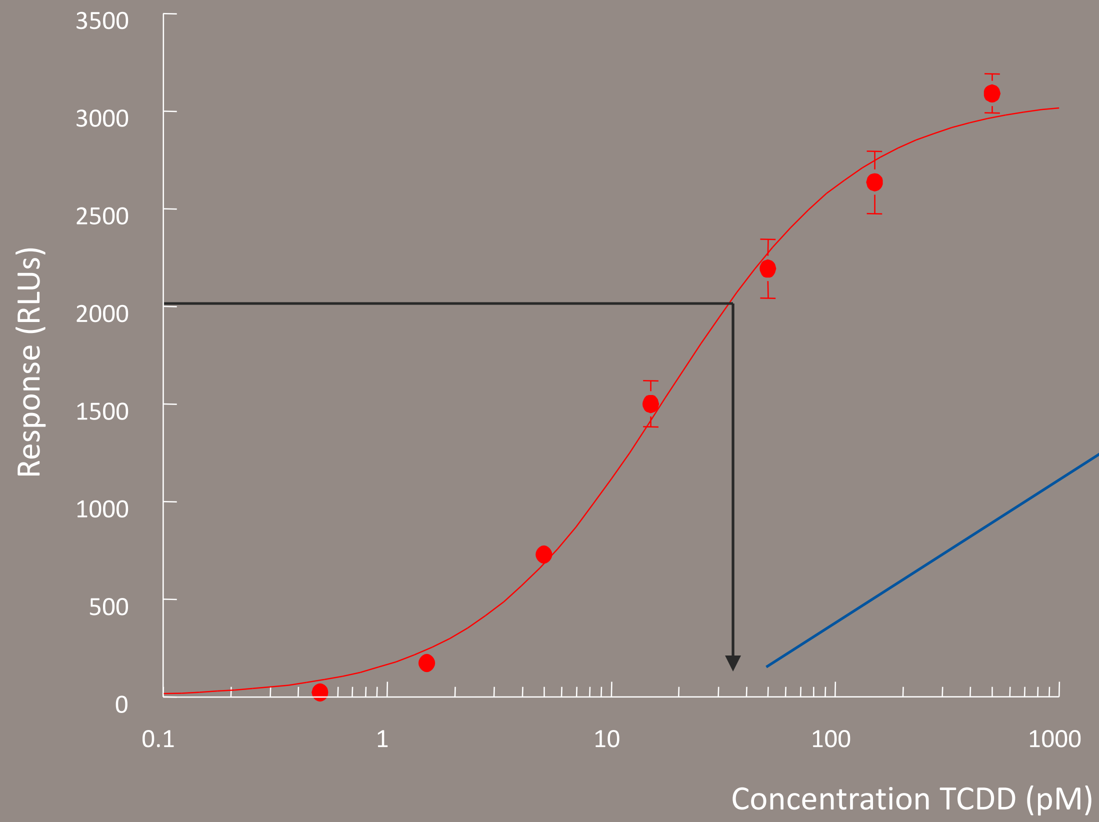




Luciferin
+
ATP

Aarts et al. 1993

Estimation of level in sample



Level in test sample

Where to look for dioxins and/or PCBs?

Sources?

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Sources of PCBs

- Used as technical mixtures and may still be present in older equipment
  - Transformers
  - Heat exchange equipment
  - E.g. Arochlor, Kanechlor, Clophen
- Also used in certain paints and sealants
  - Flame retardant

Problems due to building materials

- Use of building debris in courtyard (PCBs + dioxins)
- Leakage of roof coating to courtyard (PCBs + dioxins)
- Wood treated with pentachlorophenol (PCP +dioxins)

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Sources of dioxins (PCDD/Fs)

- Present as contaminants in e.g.:
  - PCB mixtures
  - Chlorophenols, like TriCP, PCP
  - Other chlorinated pesticides
- Kaolinic clay and other clays (ball clay, Mabele clay)
- Recycled minerals
- Formed during incineration of plastic waste (fires)
  - **So also drying with unsuitable fuels**

Burning of household waste, also at lower scale and some fires

So what to do during an incident?

- Quick risk assessment
- Inform consumers
- Find the source
- Which companies/farms affected?
- Slaughter animals or wait?

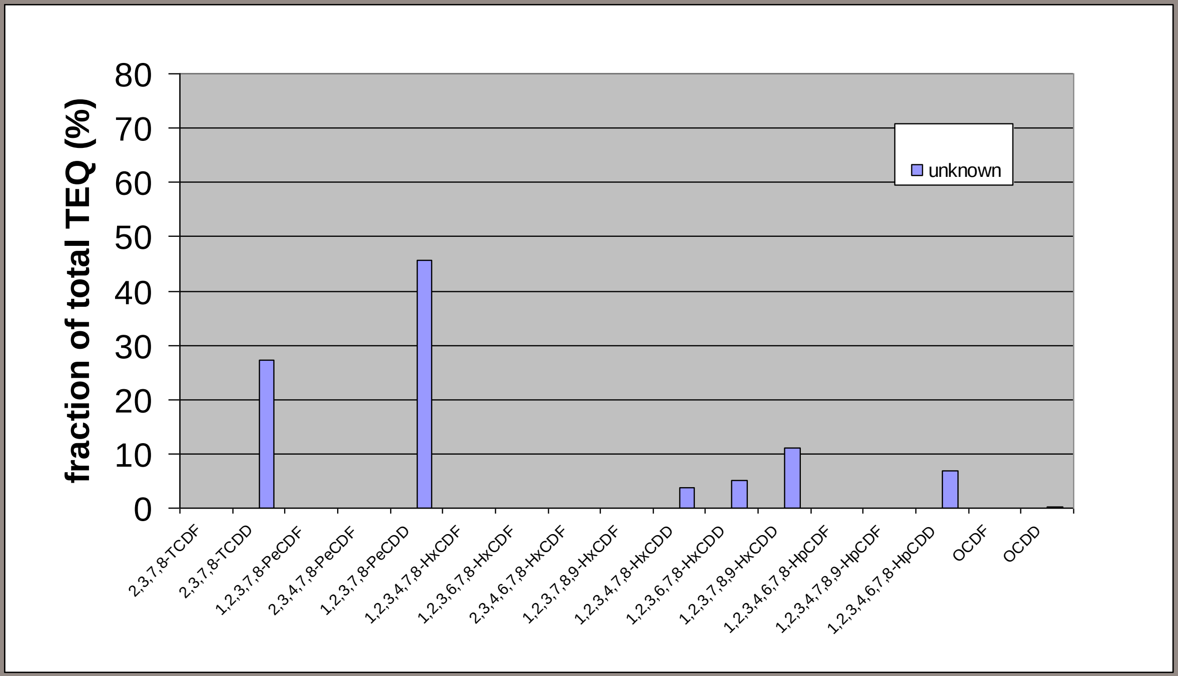
Quick risk assessment

- People worried about what they already consumed
- In some incidents exceedance of TWI
- But also good to compare with existing body burden
    - Typical value 100-200 ng TEQ
    - Performed in Irish incident (EFSA, 2008)
    - Often limited contribution, unless long duration

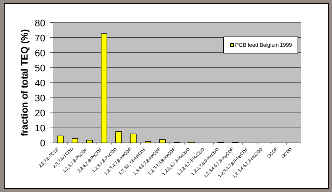
So what to do during an incident?

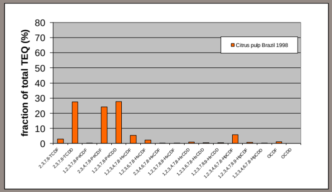
- Quick risk assessment
- Inform consumers
- Find the source
- Which companies/farms affected?
- Slaughter animals or wait?

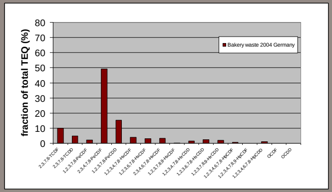
Unknown pattern in potato peels (2004)

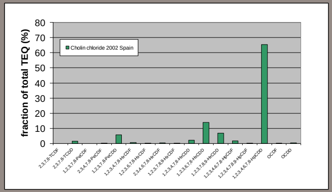


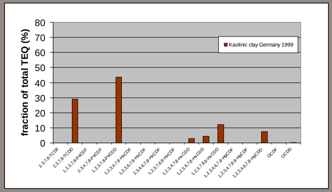
Identification source

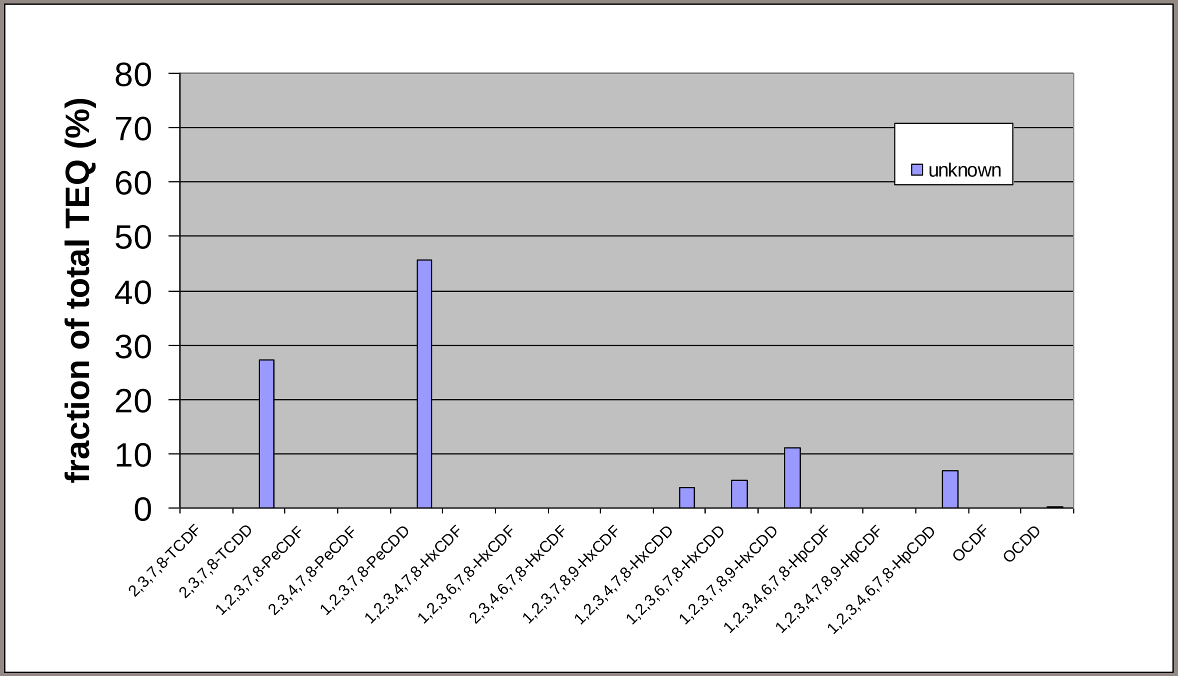




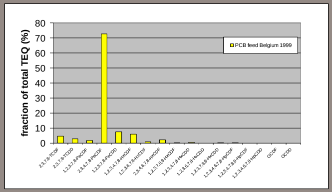


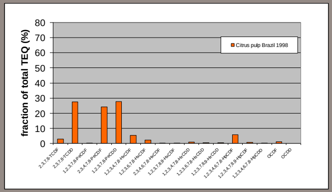


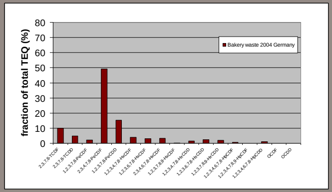


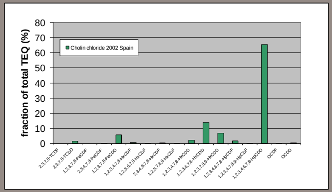


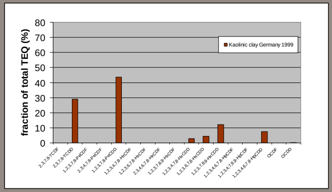
Identification source

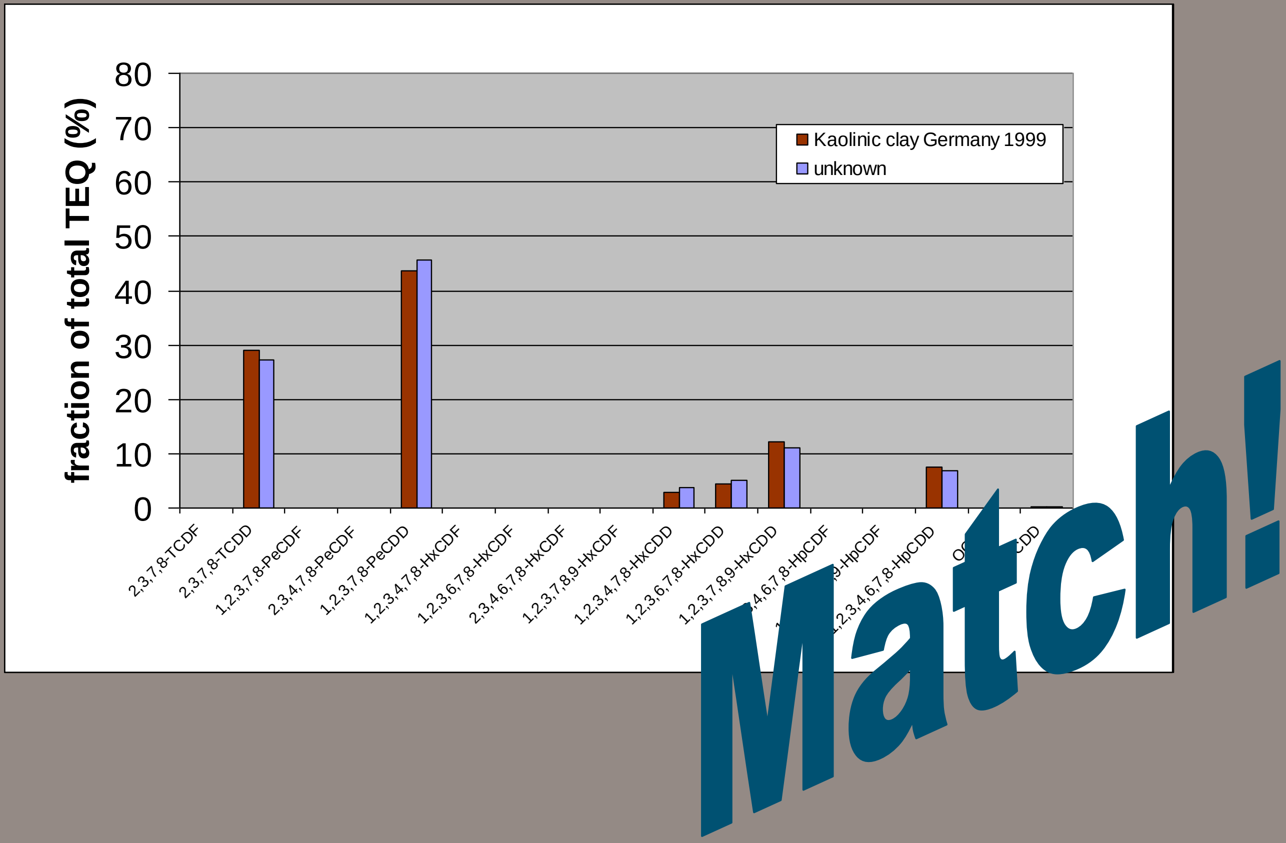








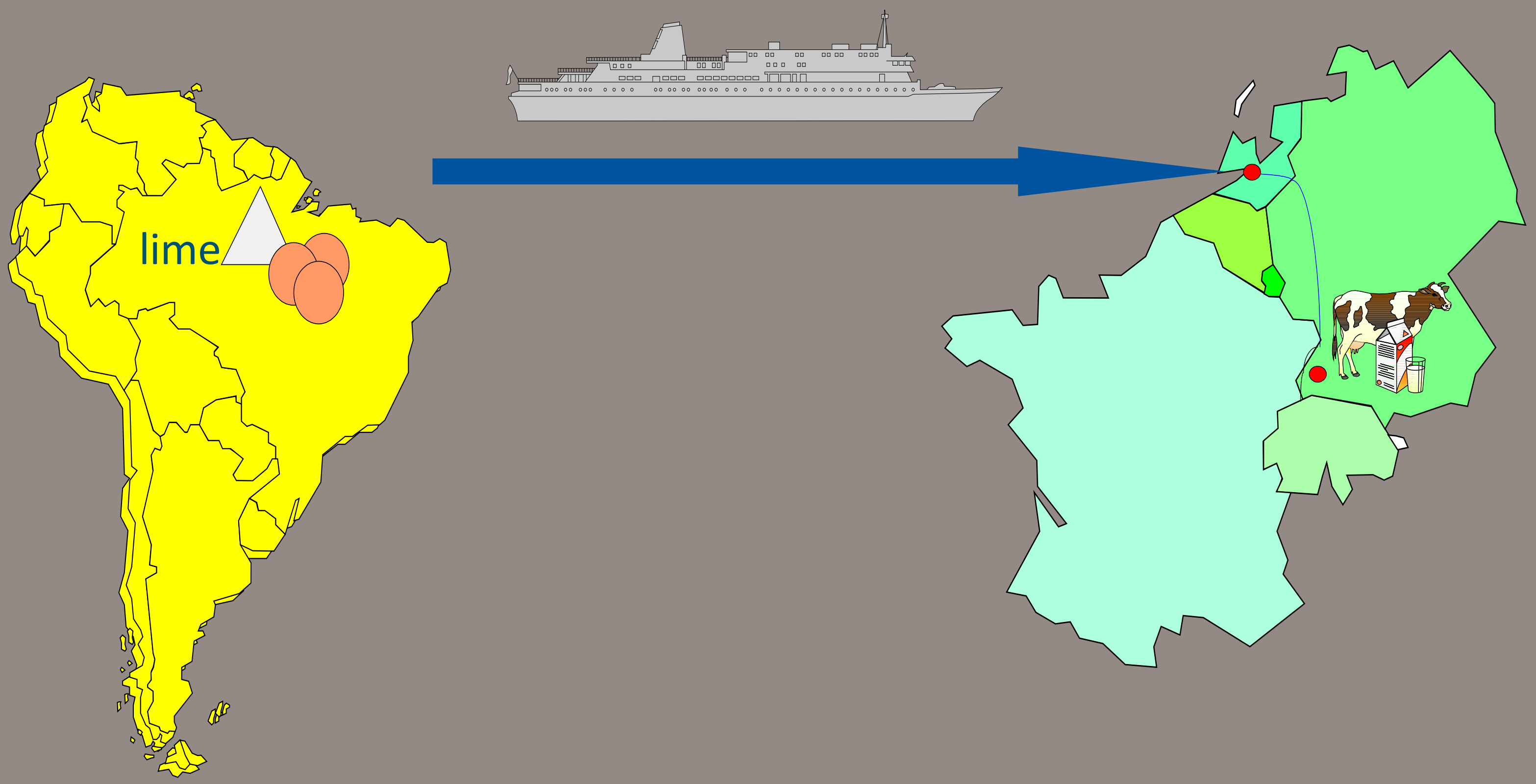




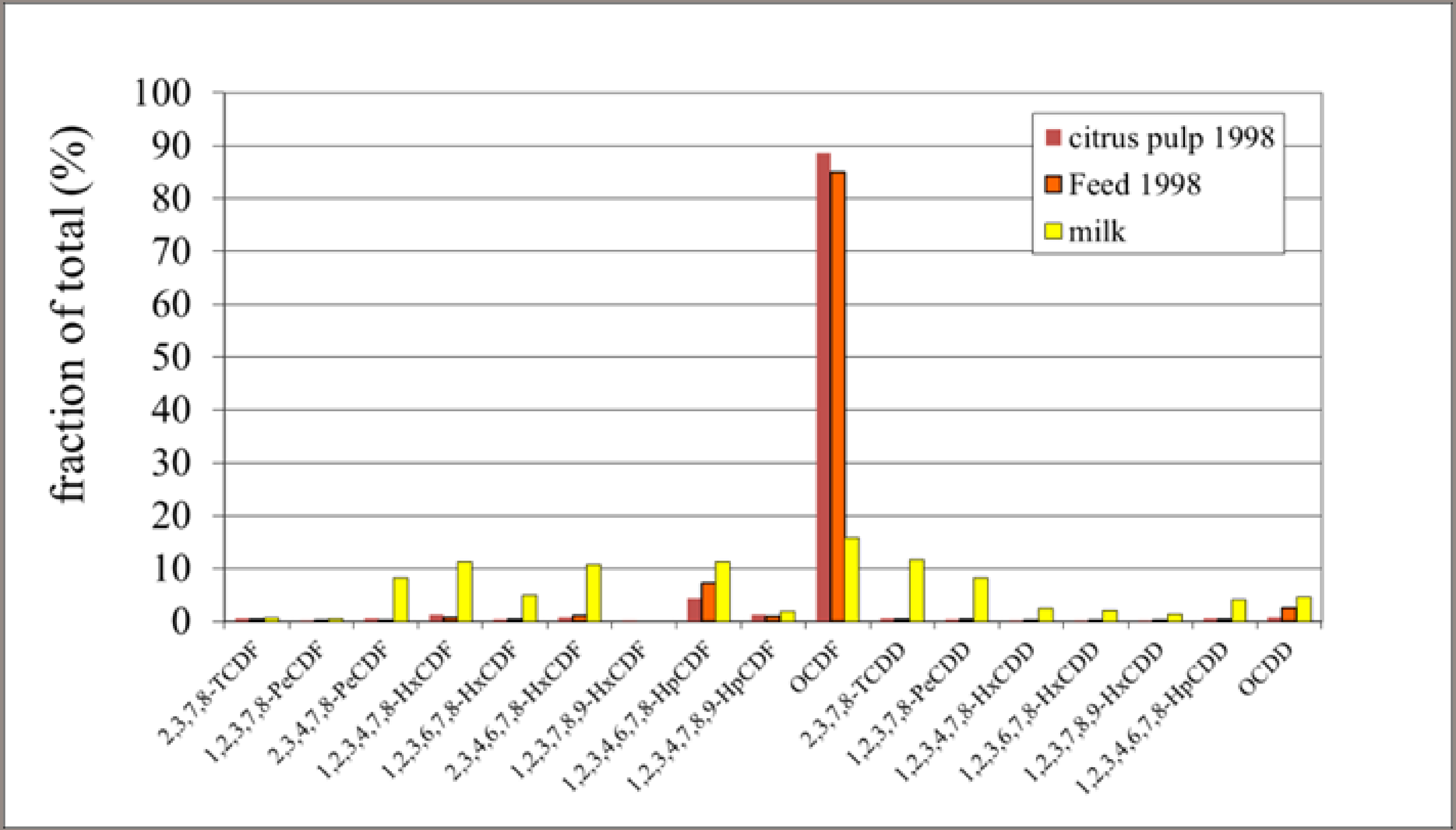
Which patterns?

- Primary patterns: so not in animal derived products
- Primary focus on dioxin patterns (PCDD/Fs)
  - Specification PCB source not always required
  - Expressed on contribution to absolute or TEQ levels?
- Patterns will change in animal derived food

Dioxins in Brazilian citrus pulp (1998)



Expression of patterns, absolute levels or ..



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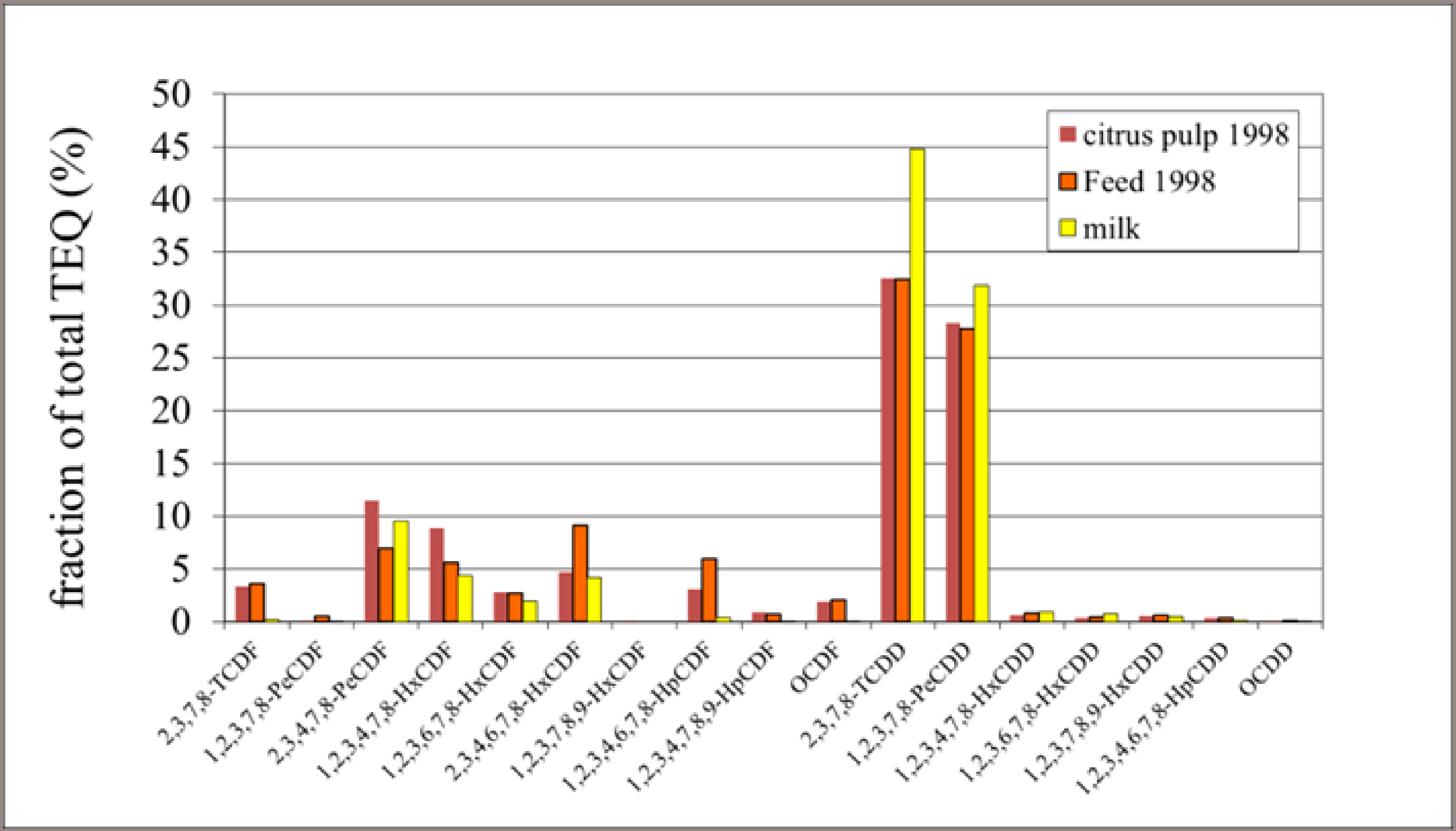
TEQ contribution?



Food and feed incidents (PCBs)

MWI milk 1989

Brazilian citrus pulp 1998

Belgian PCB fat 1999

German kaolinic clay 1999

Mozzarella Italy 2001-2004

Belgian choline chloride 2002

German bakery waste 2003

Potato peels/kaolinic clay 2004

Gelatin fat/Hydrochloric acid 2006

Indian Guar Gum 2007

Minerals (Zinc) Chile 2008

Bakery waste Ireland 2008

Organic corn Ukraine 2010

Fatty acids Germany 2011

Beet pulp Germany 2011

Free range eggs

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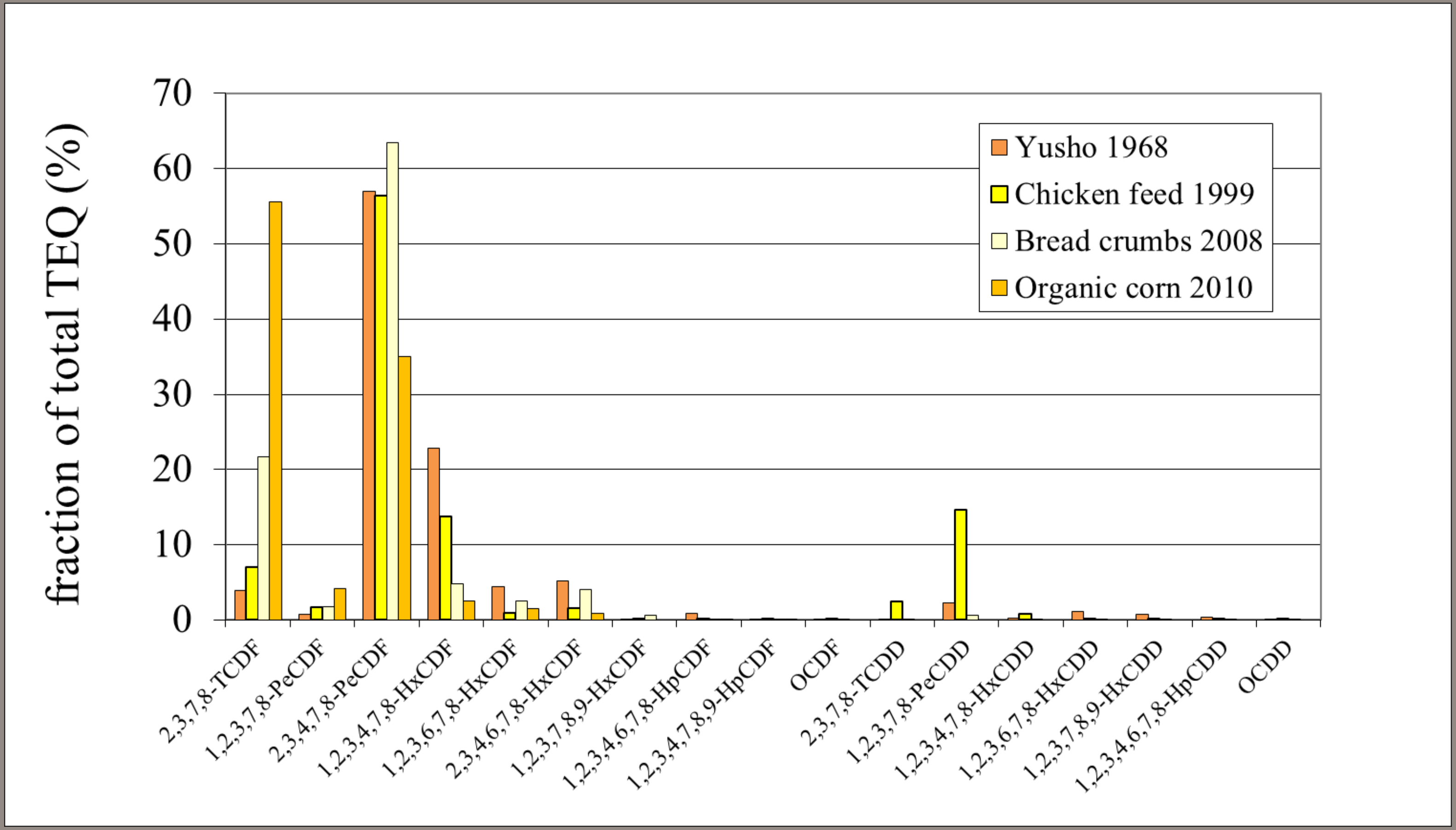
PCBs



Primarily PCDFs; pattern depends on mixture

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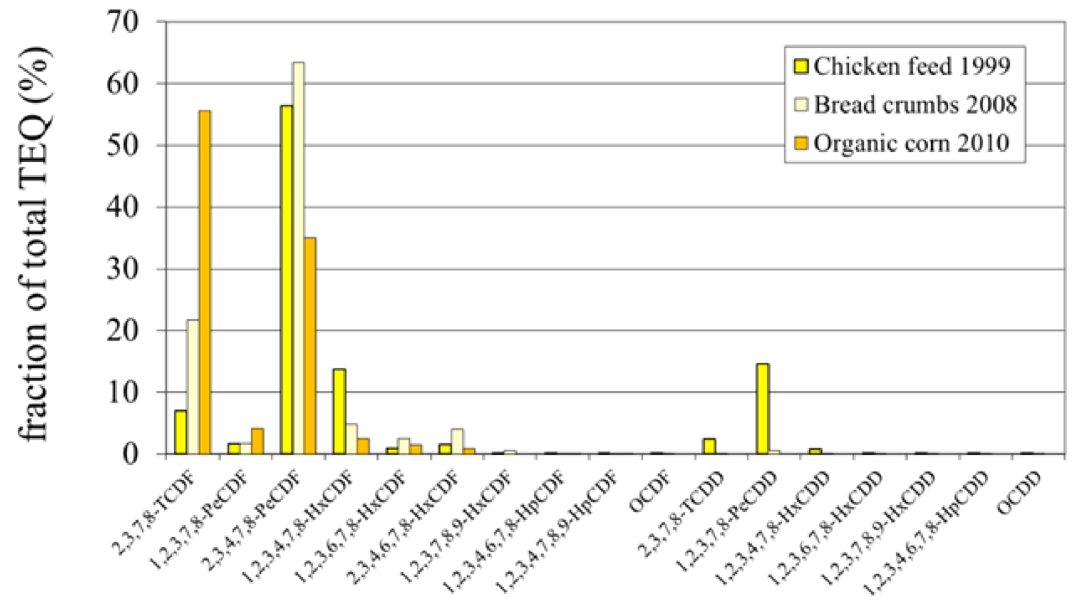
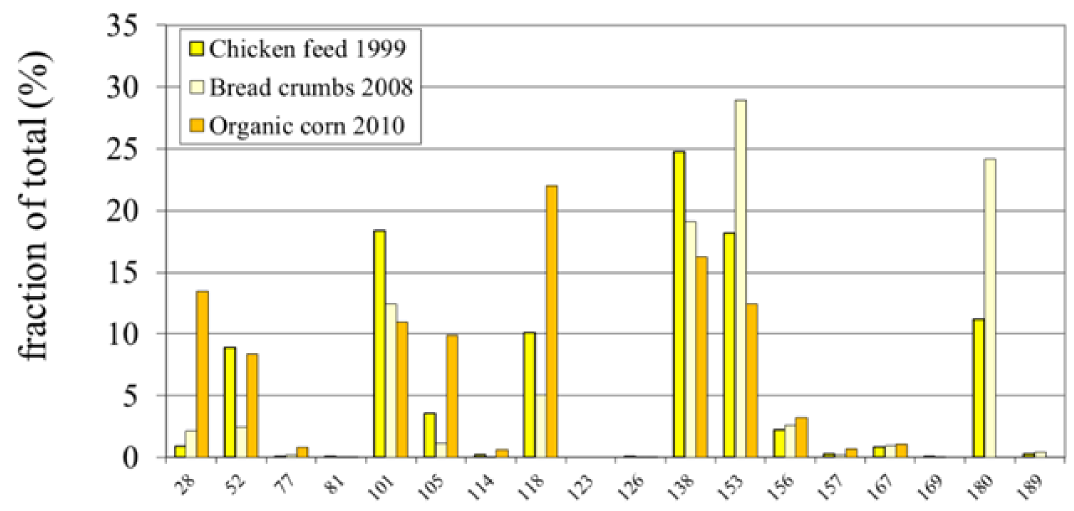
PCBs





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Food and feed incidents (chlorophenols)

MWI milk 1989

Brazilian citrus pulp 1998

German kaolinic clay 1999

Mozzarella Italy 2001-2004

Belgian choline chloride 2002

German bakery waste 2003

Free range eggs

Potato peels/kaolinic clay 2004

Gelatin fat/Hydrochloric acid 2006

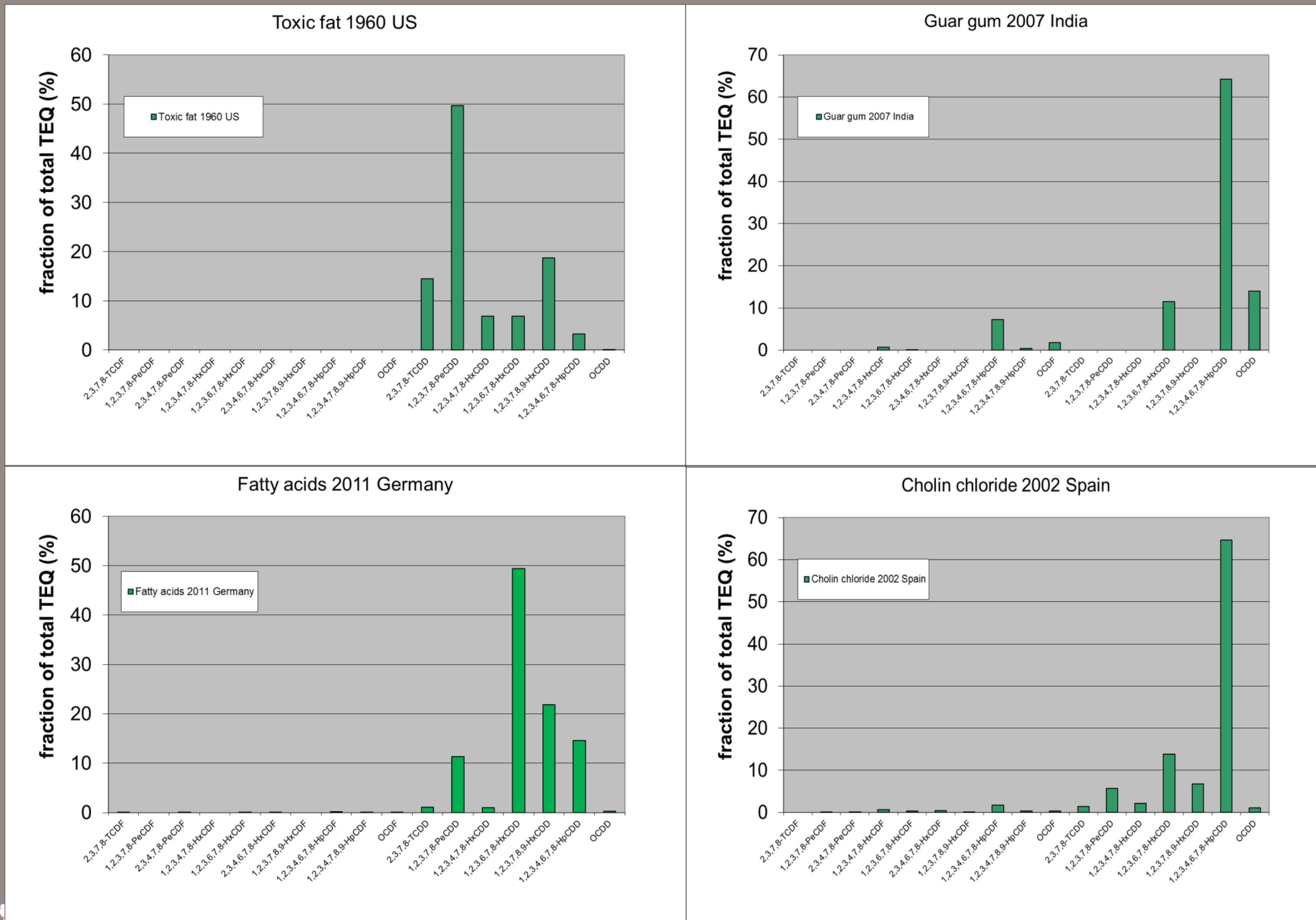
Indian Guar Gum 2007

Minerals (Zinc) Chile 2008

Fatty acids Germany 2011

Beet pulp Germany 2011

Chlorophenols (PCDDs)



Food and feed incidents (clays)

MWI milk 1989

Brazilian citrus pulp 1998

German kaolinic clay 1999

Mozzarella Italy 2001-2004

German bakery waste 2003

Free range eggs

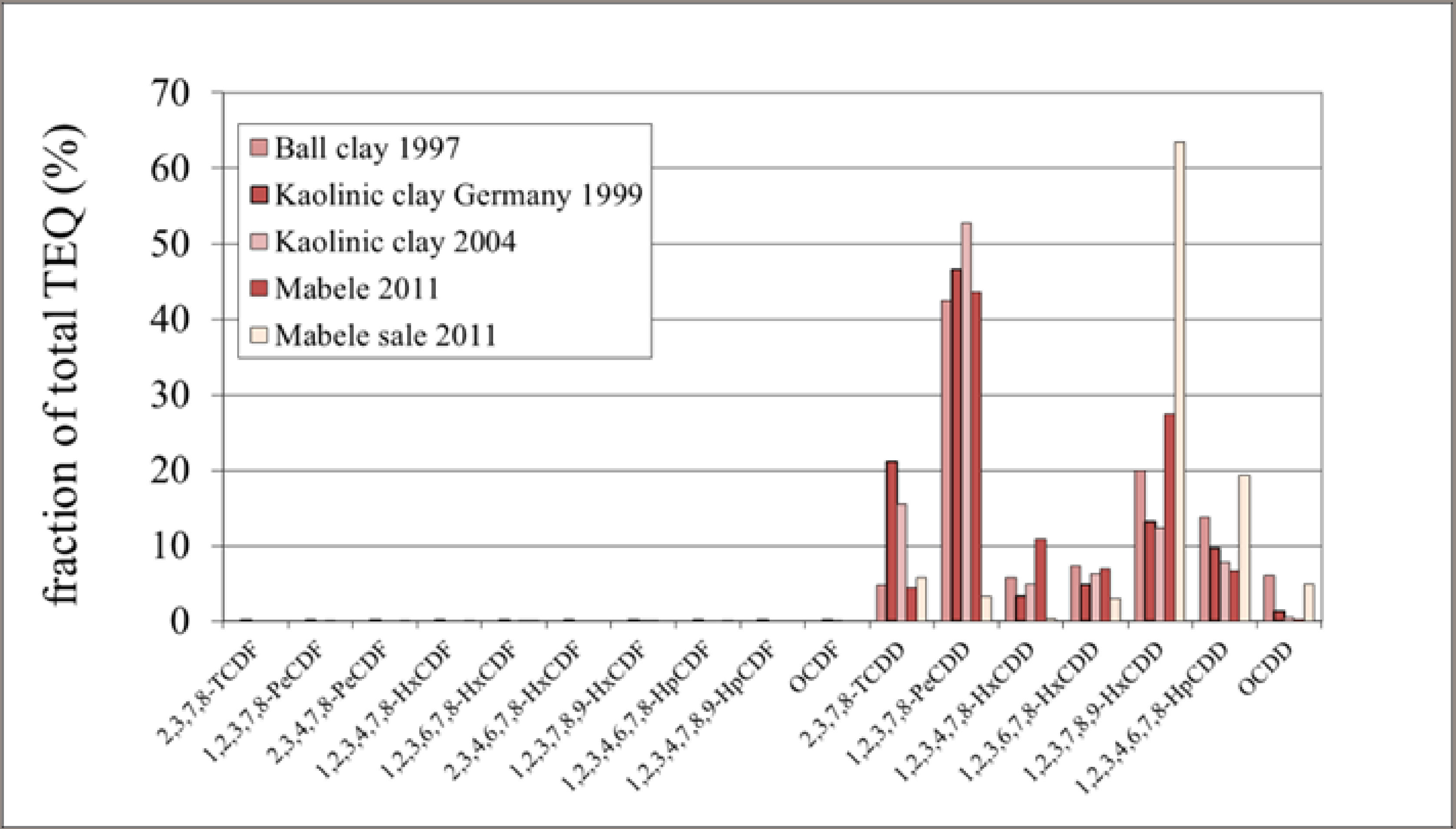
Potato peels/kaolinic clay 2004

Gelatin fat/Hydrochloric acid 2006

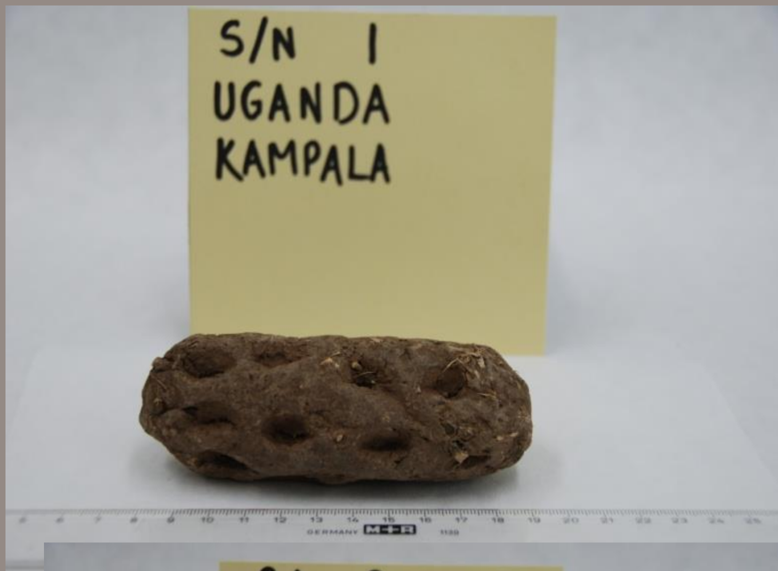
Minerals (Zinc) Chile 2008

Beet pulp Germany 2011

Clays (also PCDDs)



Pregnancy clays collected from Africa



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Risk communication

In the Netherlands advice not to use these clays

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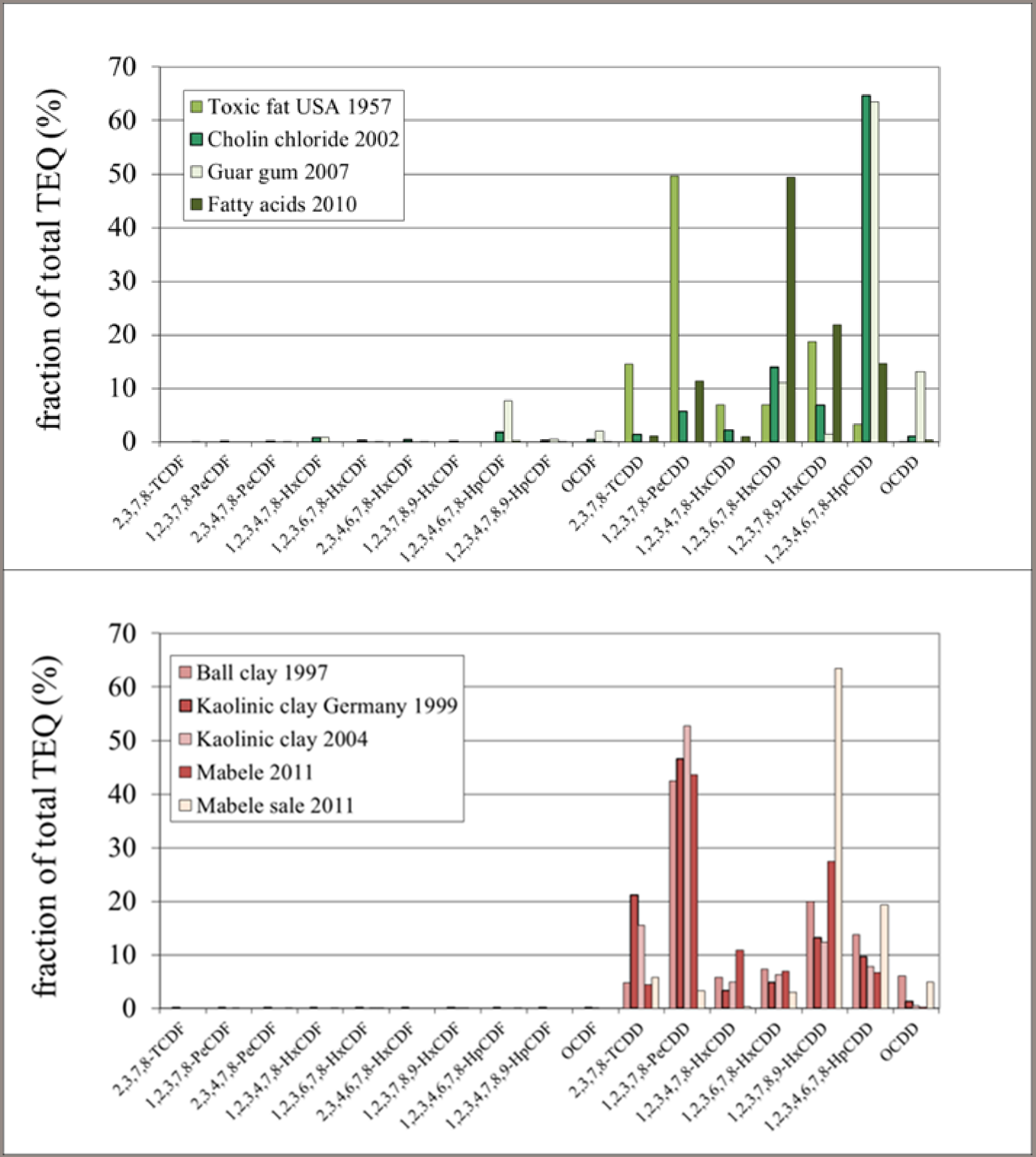
chlorophenols
vs
clays



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Food and feed incidents (minerals)

MWI milk 1989

Brazilian citrus pulp 1998

Mozzarella Italy 2001-2004

German bakery waste 2003

Free range eggs

Gelatin fat/Hydrochloric acid 2006

Minerals (Zinc) Chile 2008

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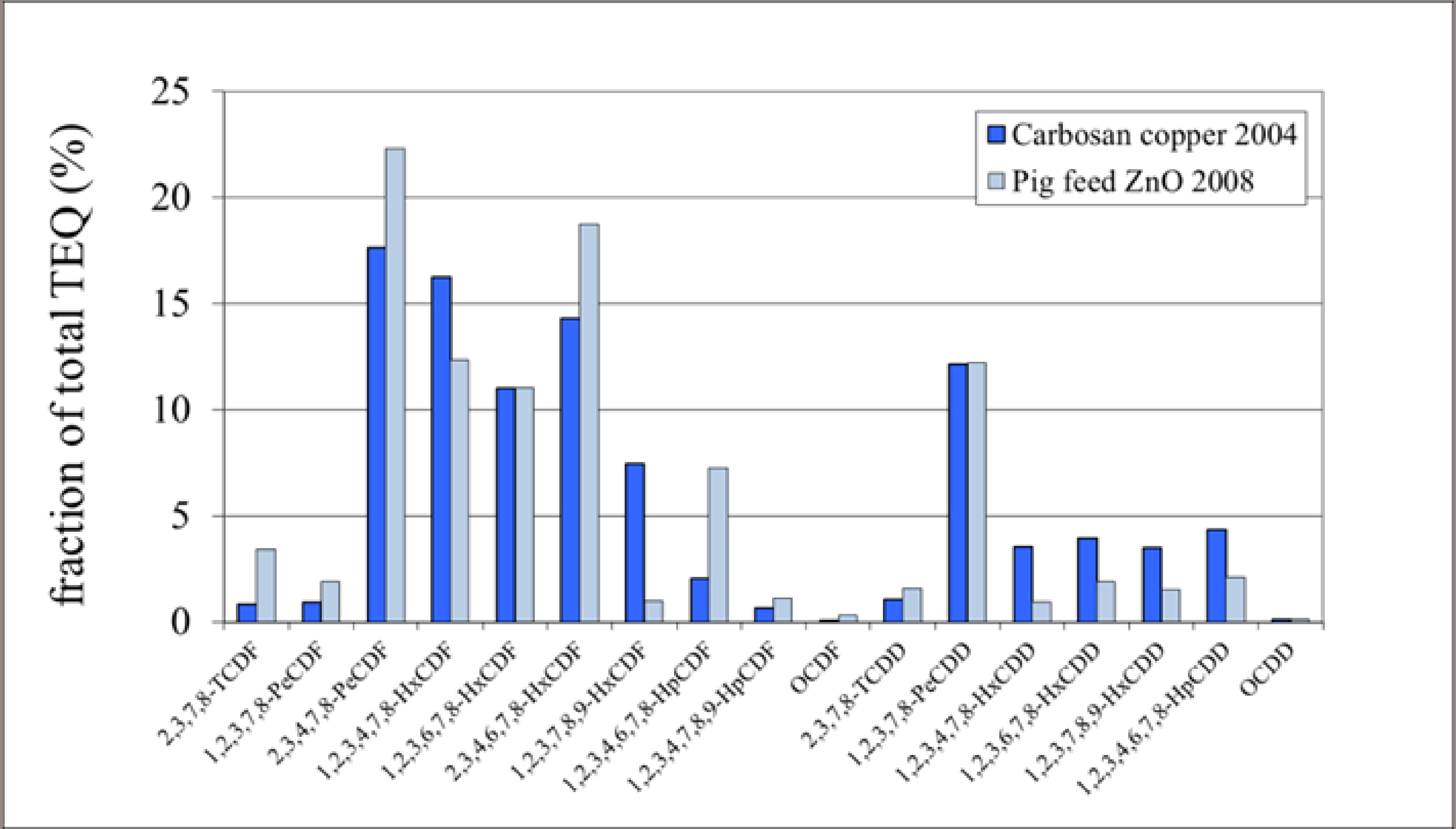
Beet pulp Germany 2011

Minerals
(dominated by PCDFs but also some PCDDs)



Food and feed incidents (burning of plastics)

MWI milk 1989

Brazilian citrus pulp 1998

Mozzarella Italy 2001-2004

German bakery waste 2003

Free range eggs

Gelatin fat/Hydrochloric acid 2006

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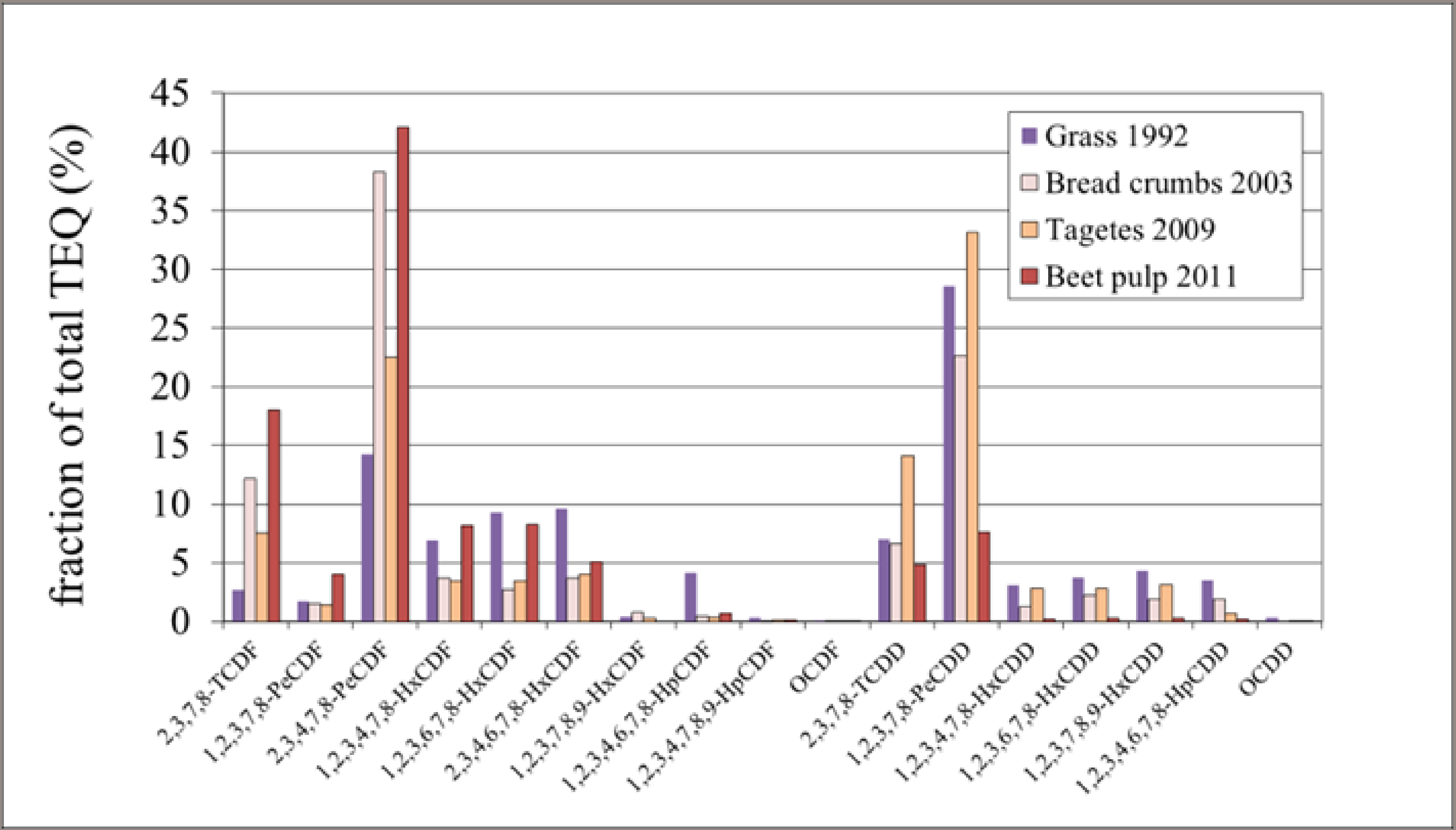
Beet pulp Germany 2011

Waste incineration (1989)

MWI

Burning (waste, fires, drying)

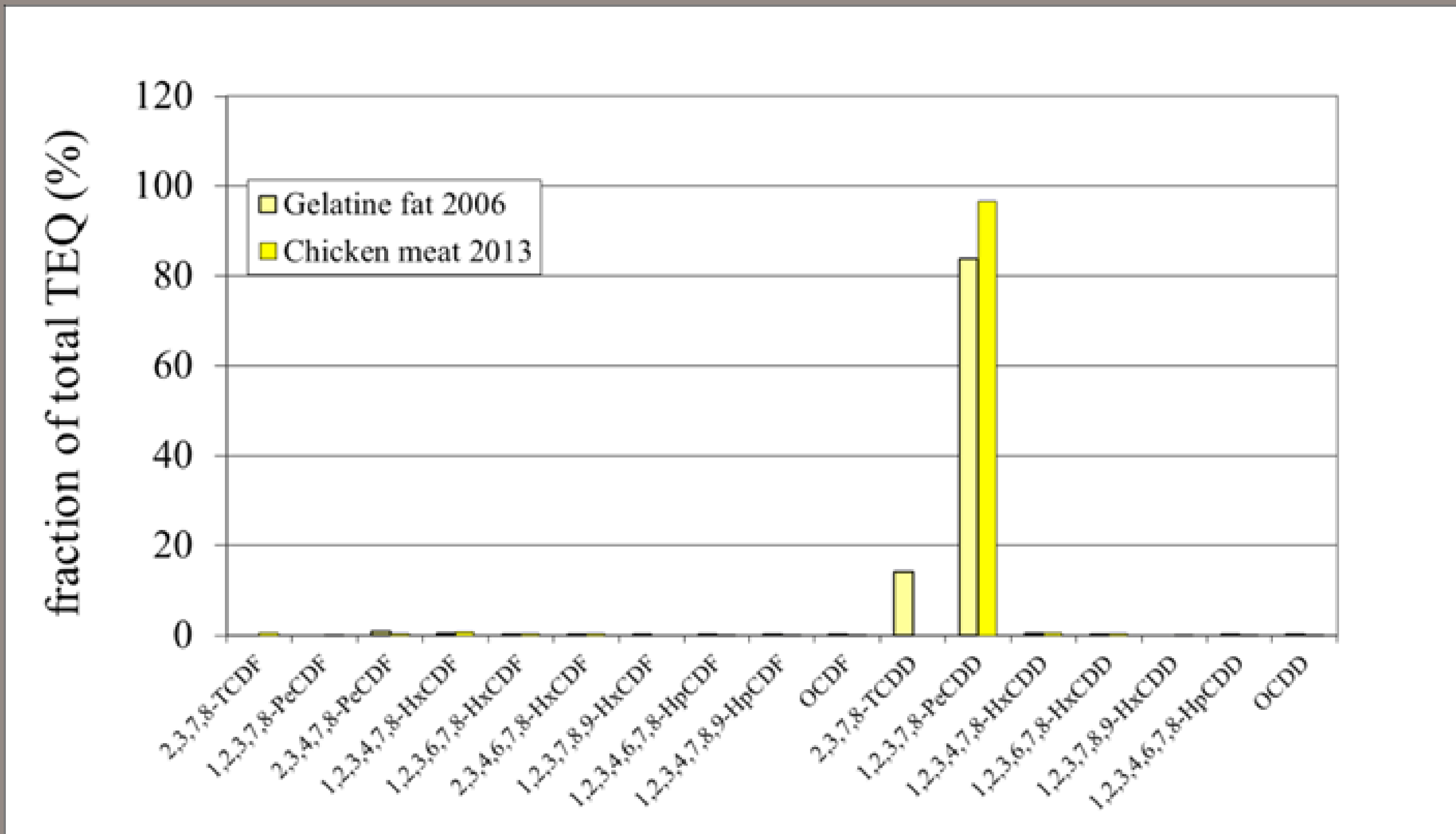


- Dominated by 23478-PeCDF/PeCDD/TCDF/ TCDD

Food and feed incidents (unknowns?)

Gelatin fat/Hydrochloric acid 2006

Unknown sources (by-product chemicals?)



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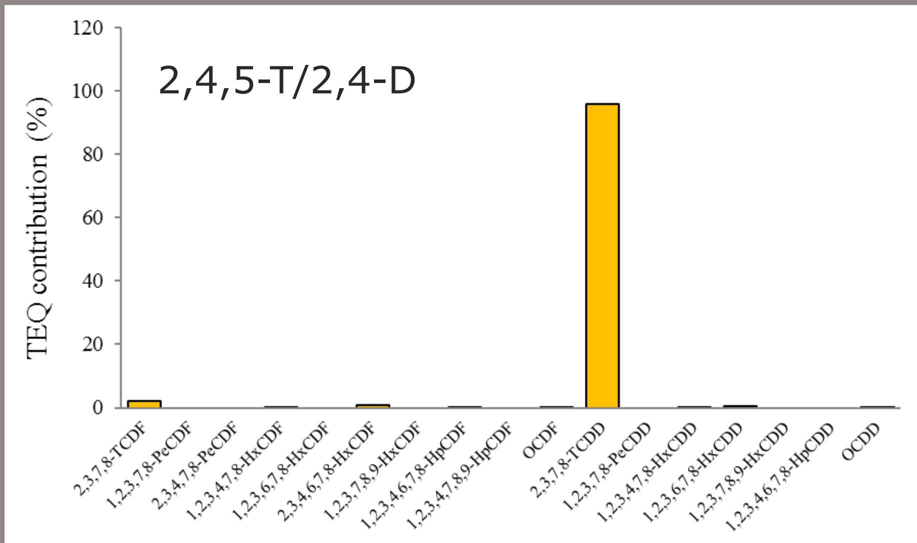
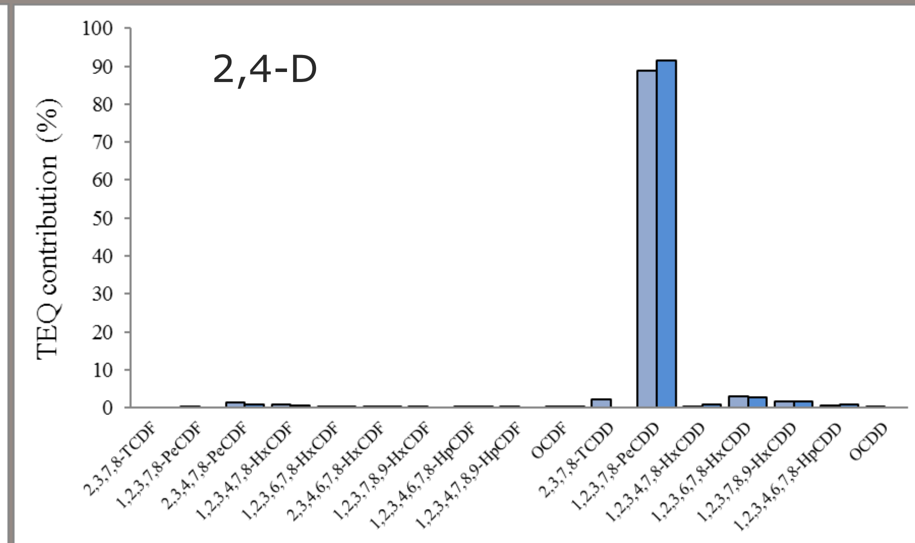
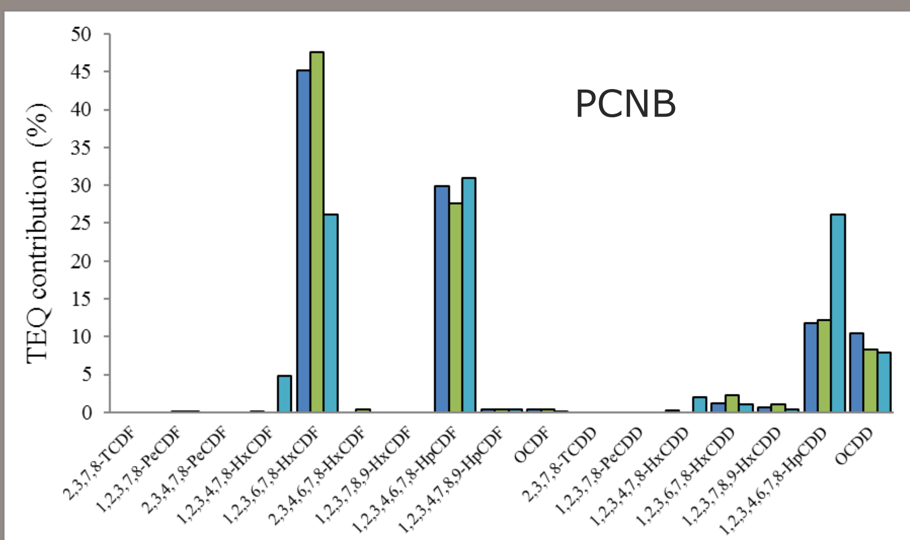
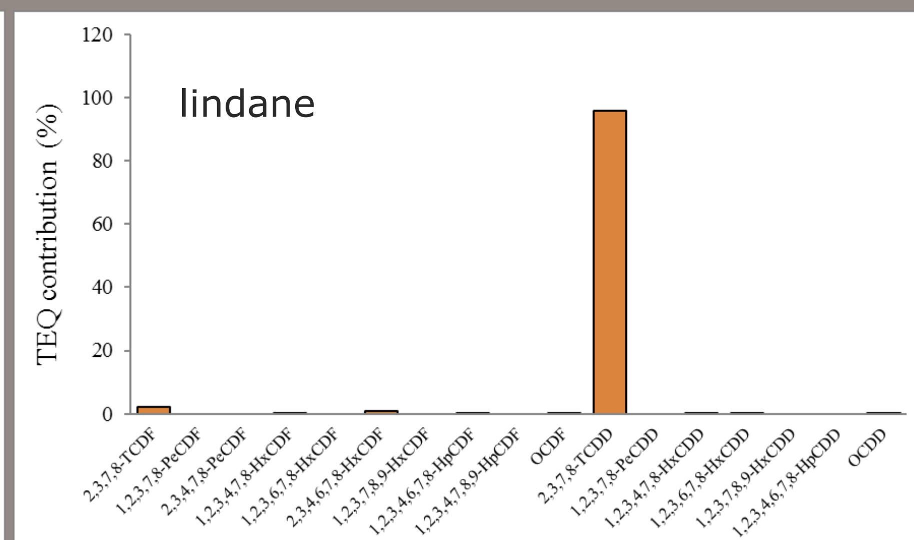
Chlorinated pesticides (food incidents?)









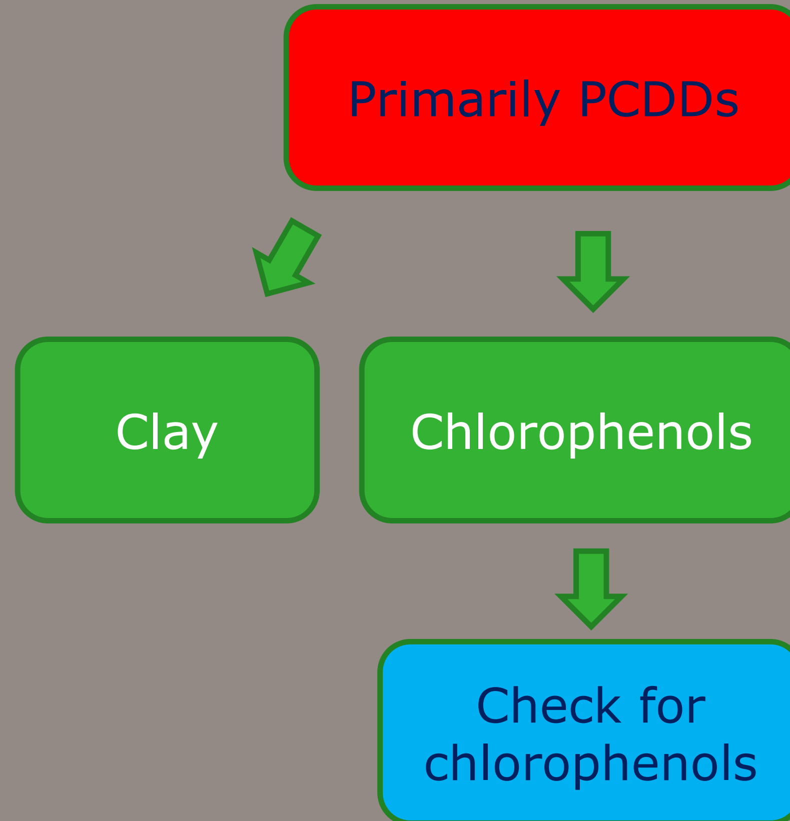
Primarily PCDFs

↓

PCBs
including burning

↓

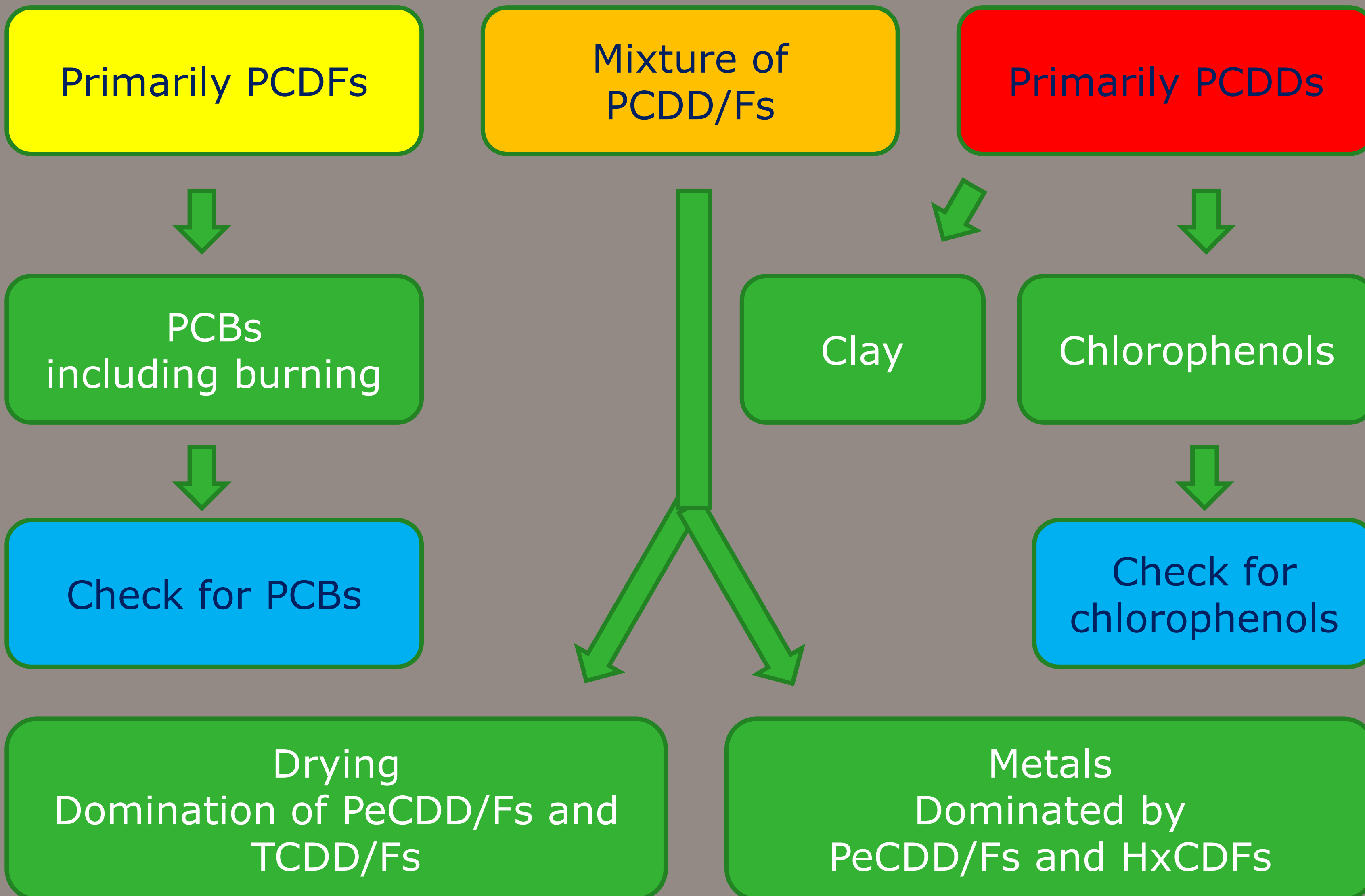
Check for PCBs


Primarily PCDDs
Clay
Chlorophenols
Check for chlorophenols

Mixture of
PCDD/Fs

Drying
Dominated by
PeCDD/Fs and TCDD/Fs

Metals
Dominated by
PeCDD/Fs and HxCDFs



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So what to do during an incident?

- Quick risk assessment
- Inform consumers
- Find the source
- Which companies/farms affected?
- Slaughter animals or wait?

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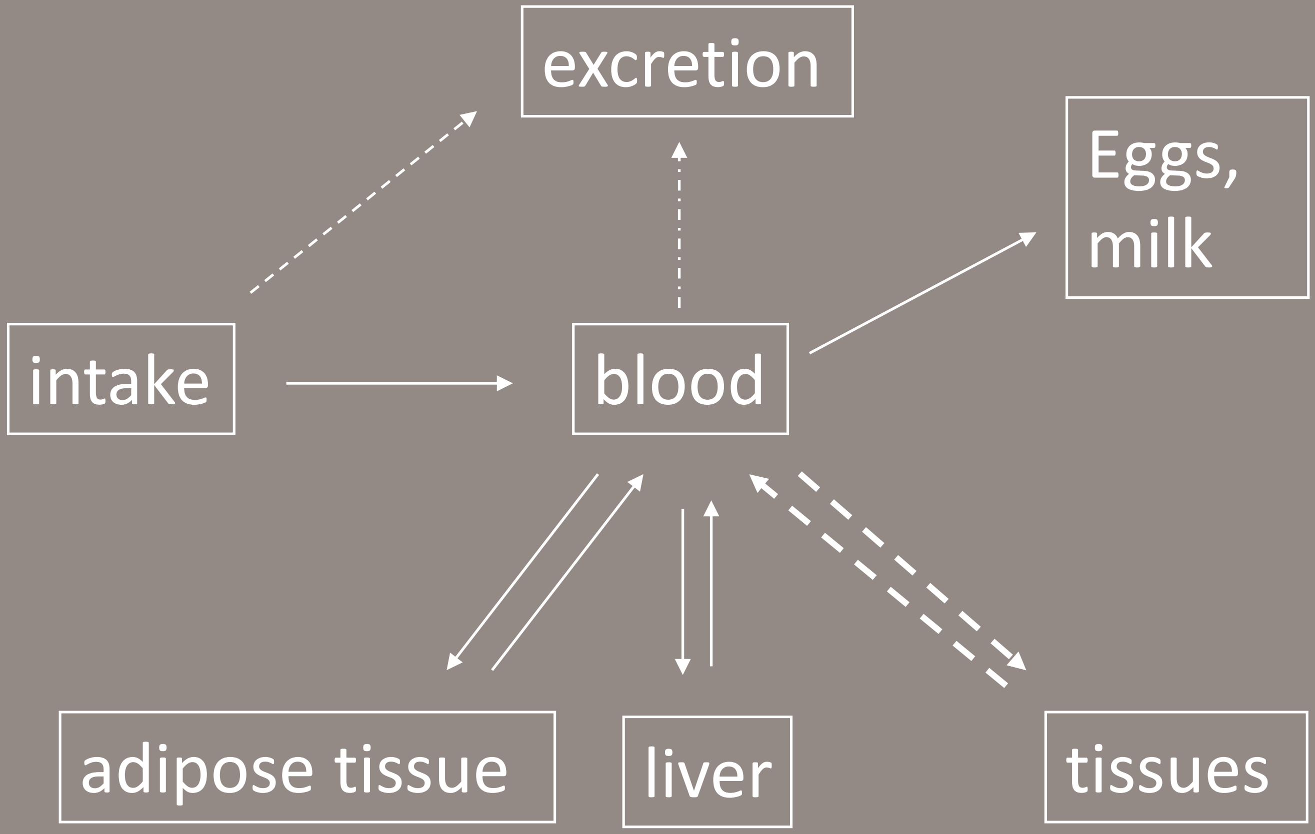
Behaviour in animals

- PCDD/Fs and DL-PCBs accumulate in tissues
    - According to fat content
    - Except for liver (binding to enzymes, sequestration)
- Are excreted in eggs and milk
- Some differences between congeners
- Use of PBK models to predict increase/decrease levels

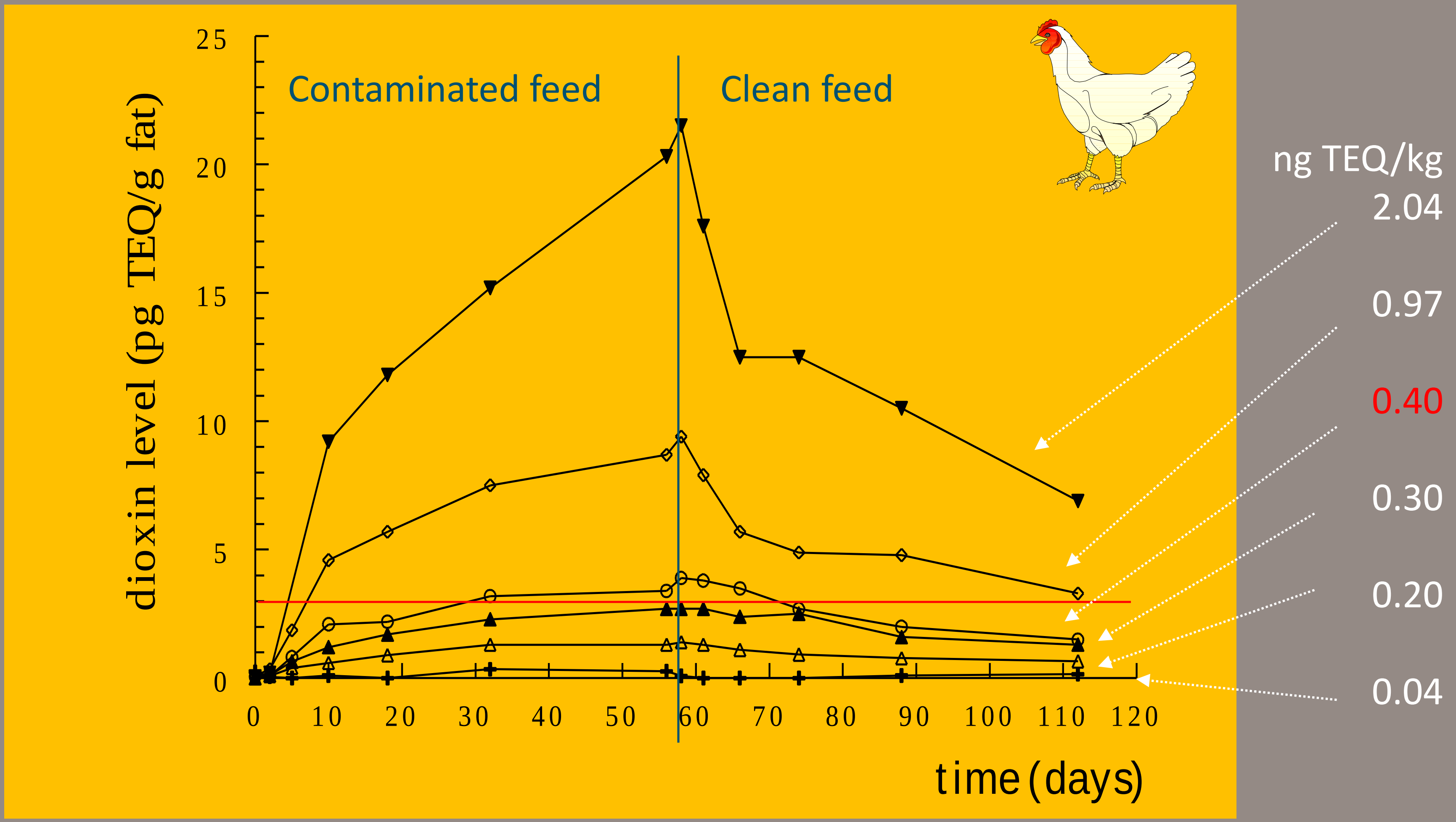
Kinetics in food producing animals



Laying hen study (RIKILT/ASG/RIVM)

- Mixture of PCDD/Fs and PCBs added to feed at different levels
- Long exposure (56 d) and depletion periods (56 d)
- Both eggs and meat analyzed
- Modelling by RIVM
- Papers
    - Hoogenboom et al. 2006
    - Van Eijkeren et al. 2006

Transfer dioxins from feed to eggs



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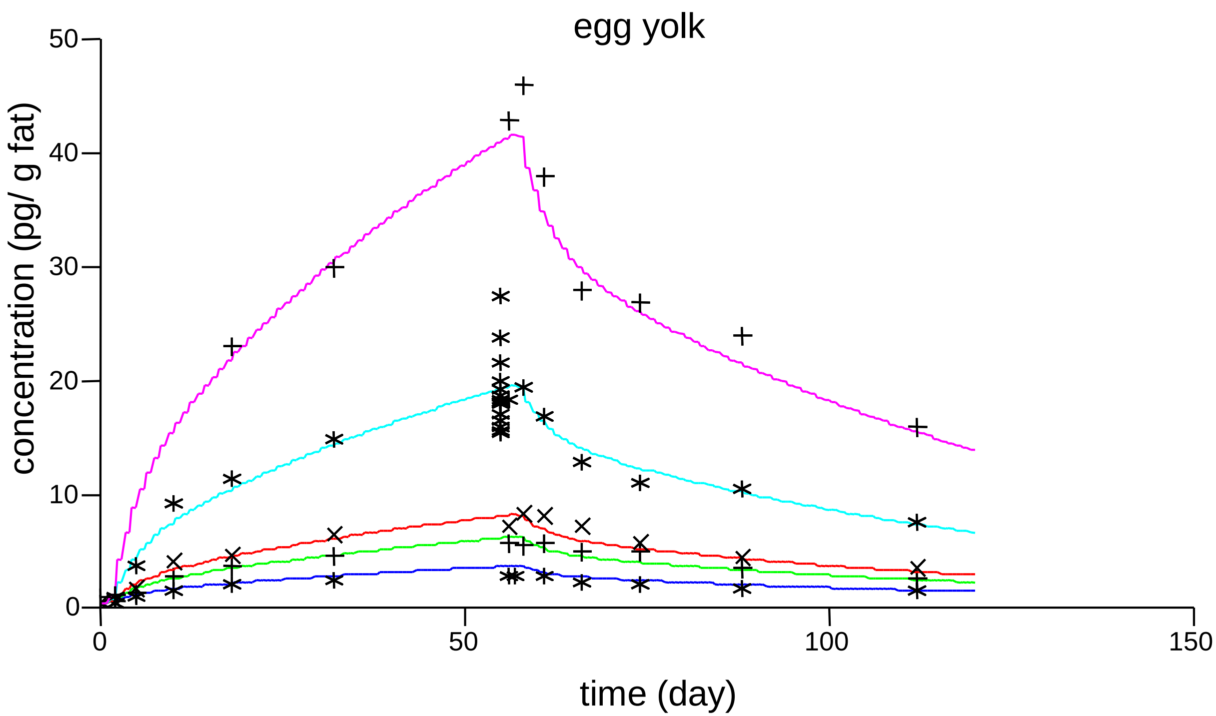
PBK modelling (sum dioxins, dl-PCBs in TEQ)



TEQ based models, also for milk

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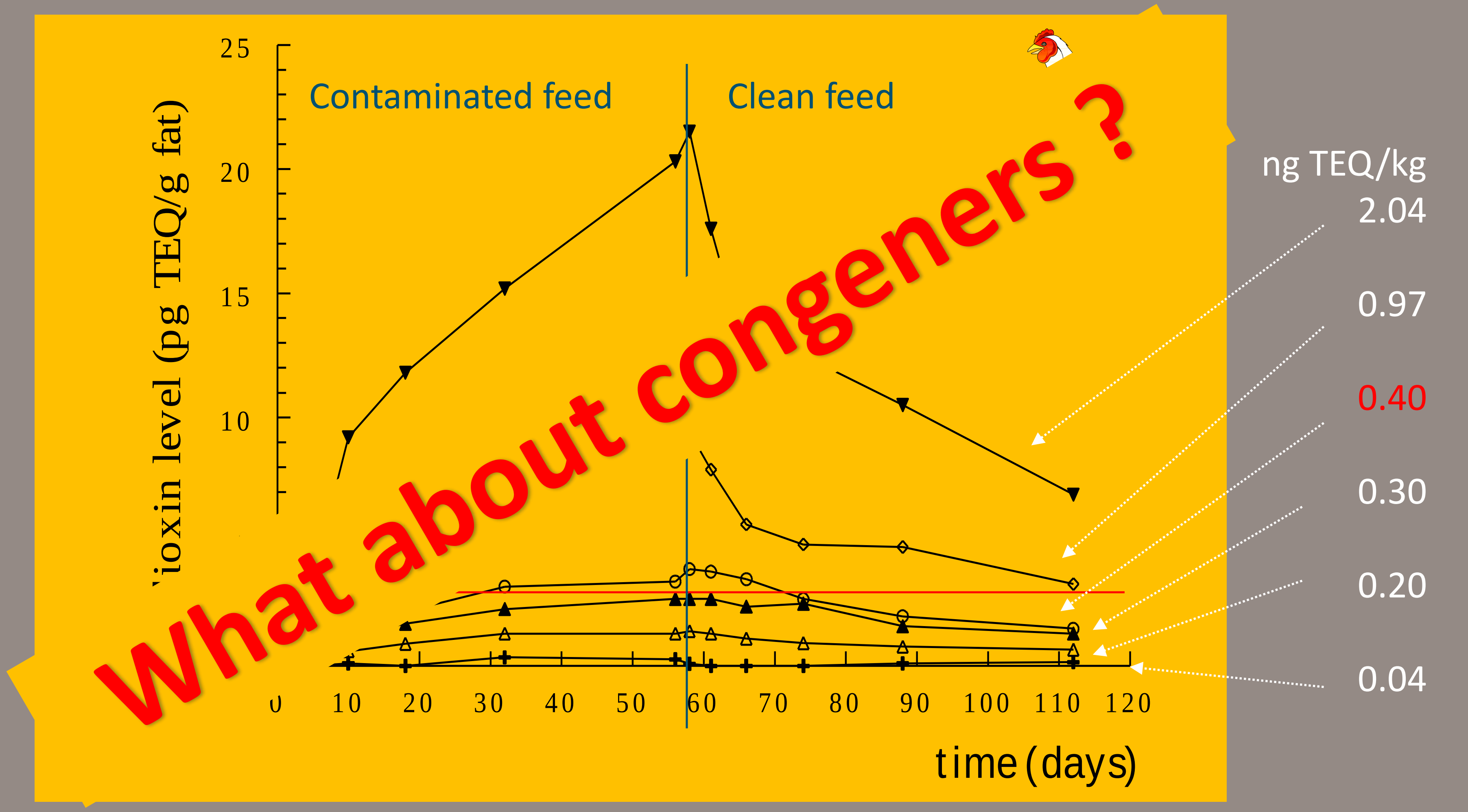
Transfer dioxins from feed to eggs



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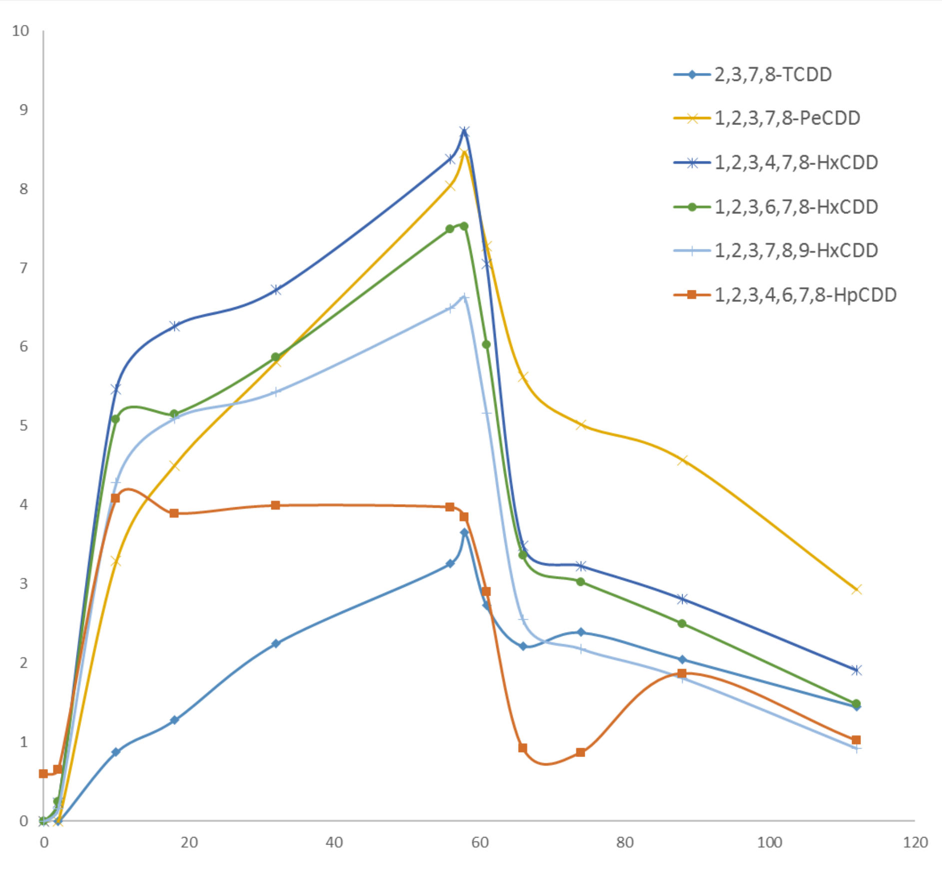
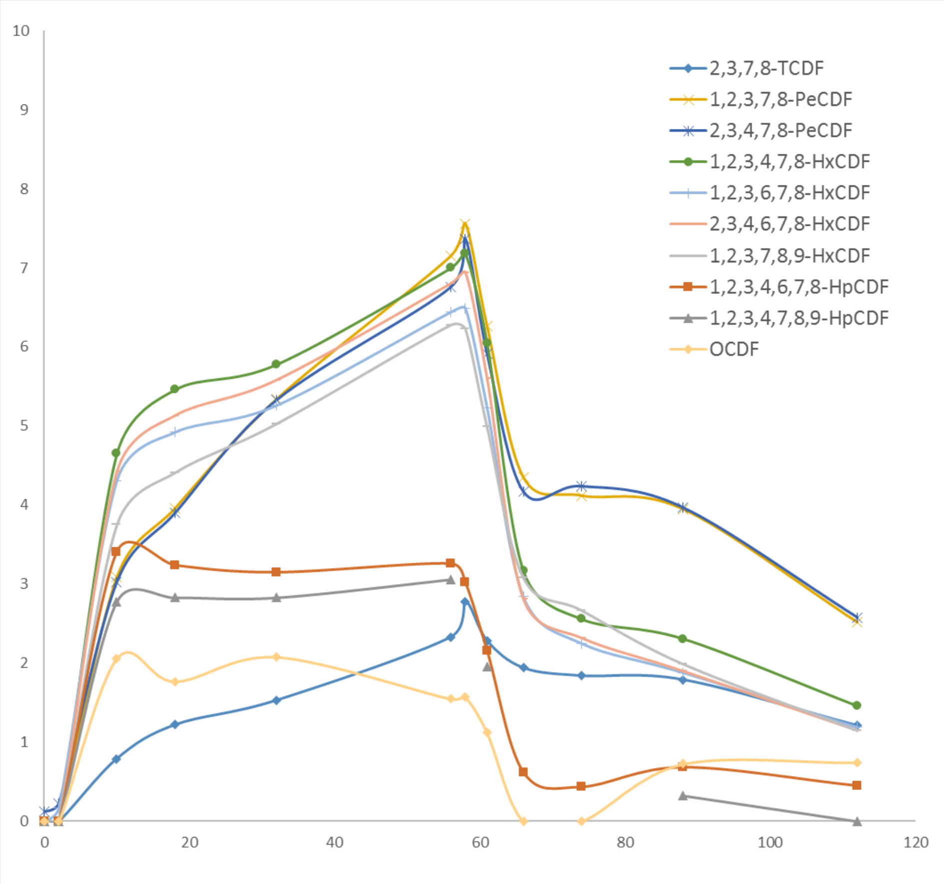
Laying hens: individual congeners





- Levels in feed differed: TCDD/F 2.5x lower, OCDD/F 2x higher

Congener-specific modelling

Dioxin patterns egg

Contamination level feed (ng/kg feed) Select CSV file or add values directly below. Run in Chrome or Firefox

Download CSV template

Browse... No file selected

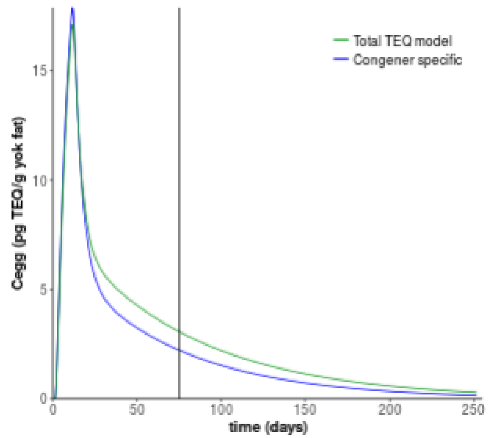
Exposure time (days)

10

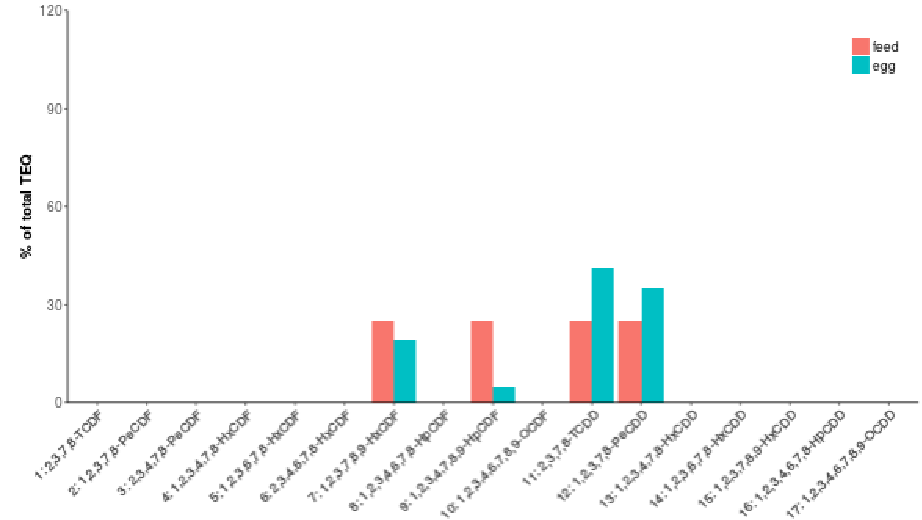
Feed intake (kg dm/day)

0.116

Calculate Reset



0 75 250



1: 2,3,7,8-TCDF

0

2: 1,2,3,7,8-PeCDF

0

3: 2,3,4,7,8-PeCDF

0

4: 1,2,3,4,7,8-HxCDF

0

5: 1,2,3,6,7,8-HxCDF

0

6: 2,3,4,6,7,8-HxCDF

0

7: 1,2,3,7,8,9-HxCDF

10

8: 1,2,3,4,6,7,8-HpCDF

0

9: 1,2,3,4,7,8,9-HpCDF

100

10: 1,2,3,4,6,7,8,9-OCDF (transfer to egg not available yet)

0

11: 2,3,7,8-TCDD

1

12: 1,2,3,7,8-PeCDD

1

13: 1,2,3,4,7,8-HxCDD

0

14: 1,2,3,6,7,8-HxCDD

0

15: 1,2,3,7,8,9-HxCDD

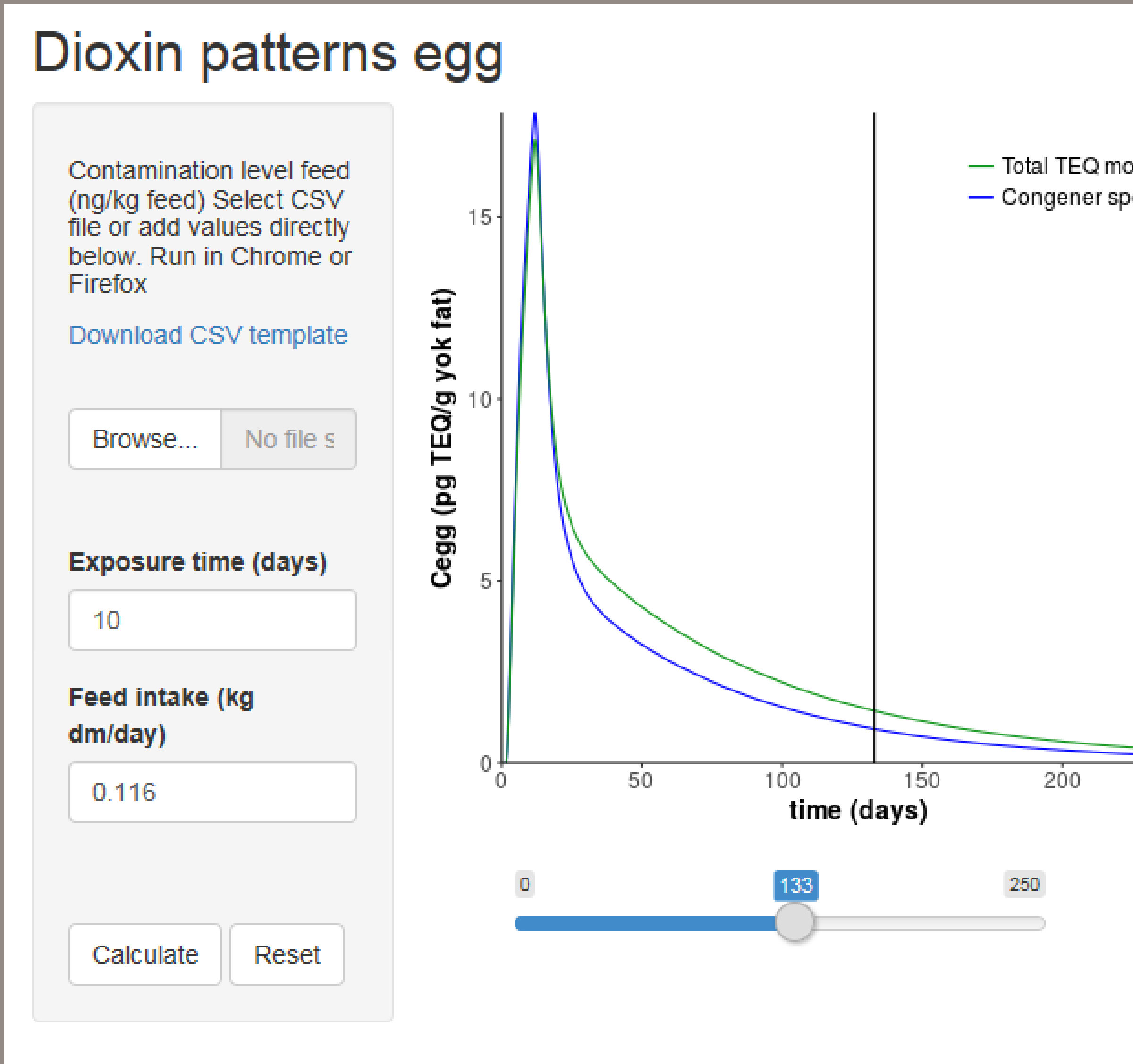
0

16: 1,2,3,4,6,7,8-HpCDD (transfer to egg not available yet)

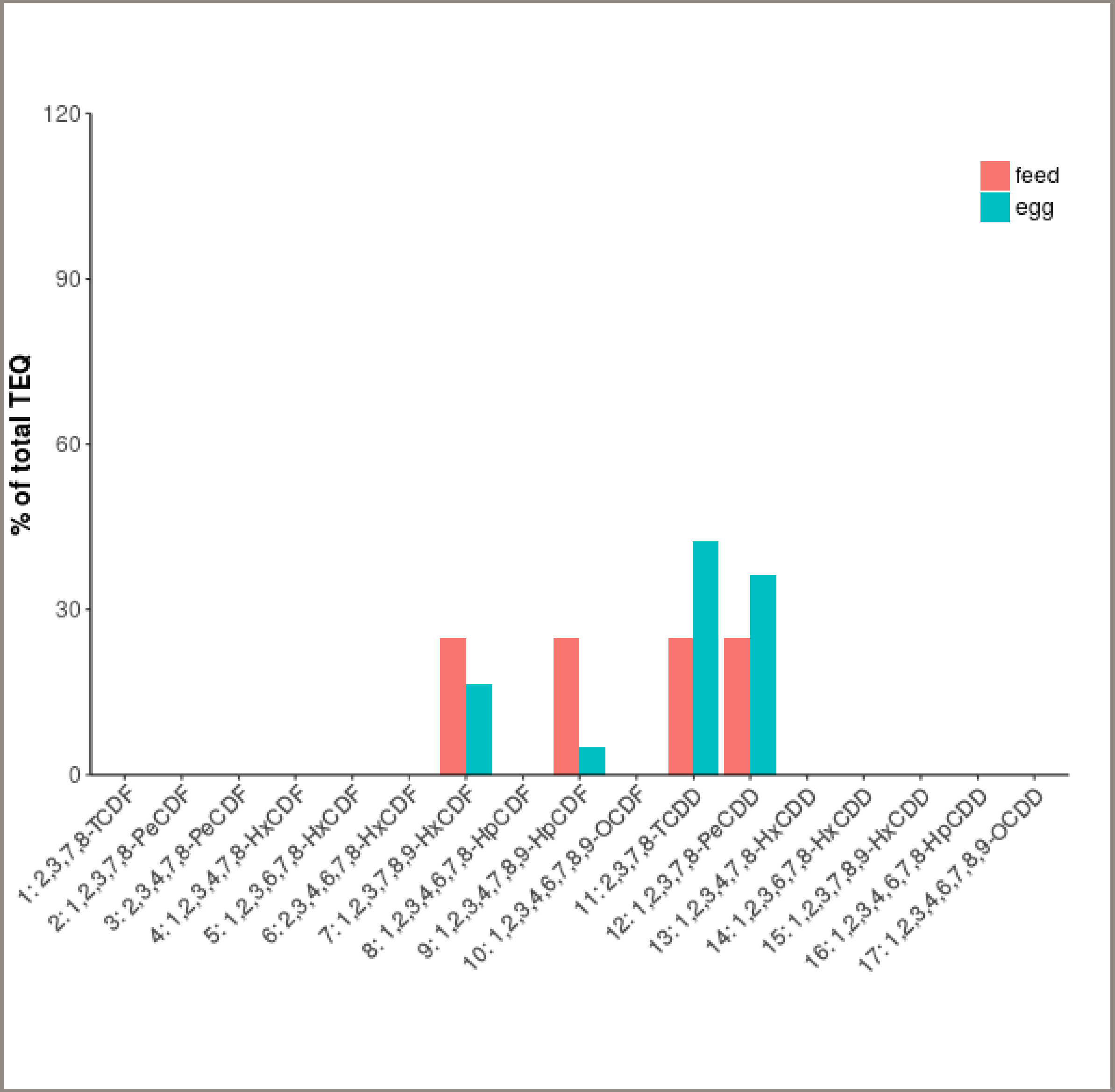
0

17: 1,2,3,4,6,7,8,9-OCDD (transfer to egg not available yet)

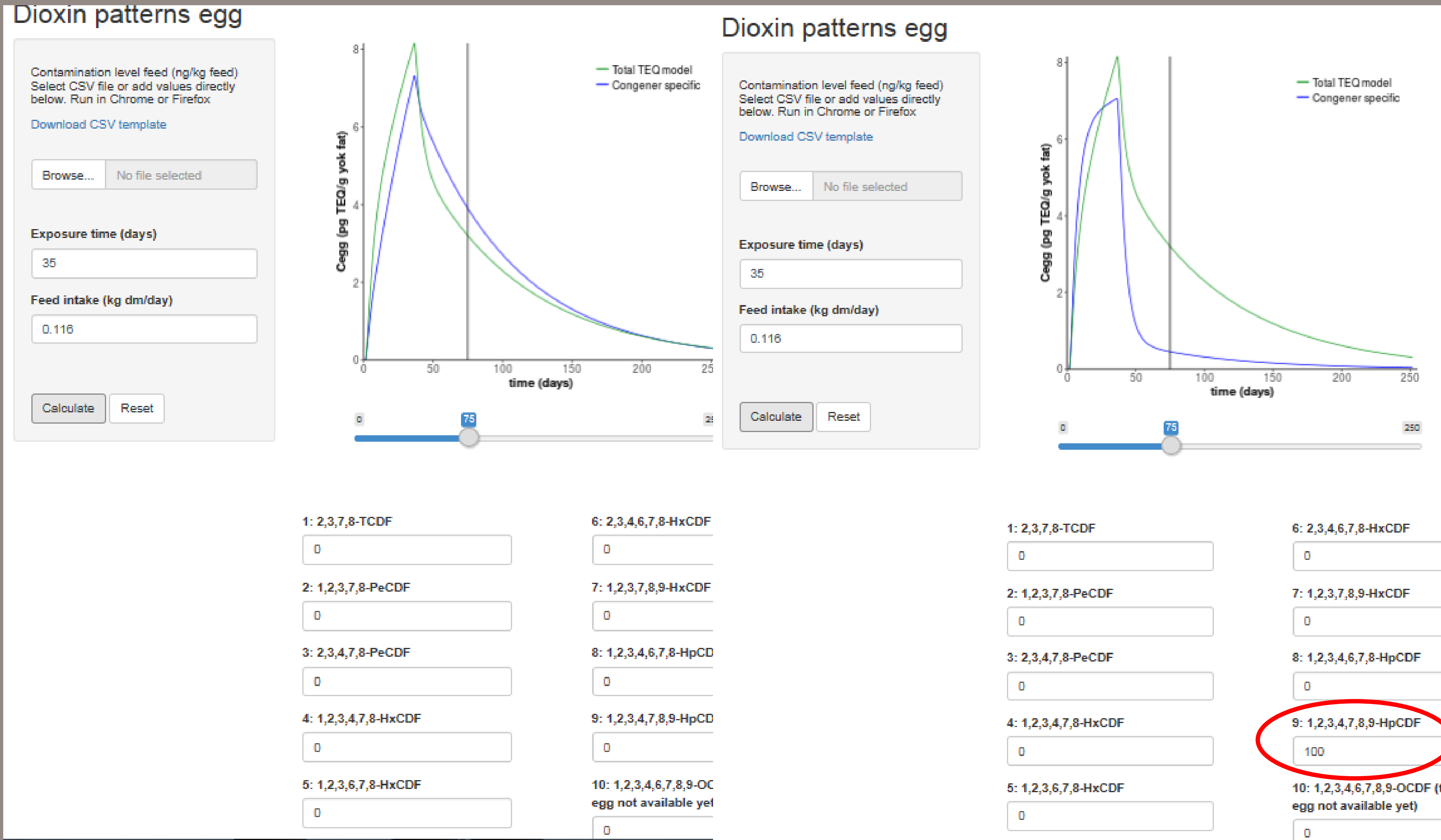
0



- Four congeners at similar TEQ levels
  - TCDD
  - PeCDD
  - HxCDF
  - HpCDF



Pattern at day 133



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Future plans

- Extend modelling
  - Add DL-PCBs and NDL-PCBs to laying hens
  - Extend to dairy cows
  - Extend to other species, like pigs, sheep, broilers
  - Extend to other compounds, also non-persistent ones
- Make it available for others through website

Limits for food and feed



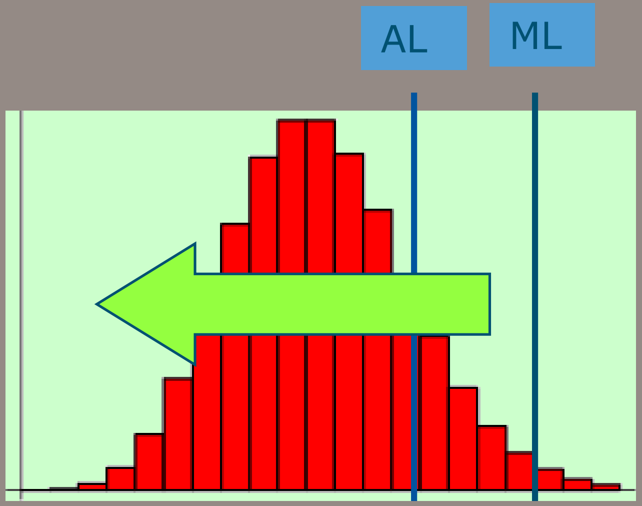

Limits for food and feed

- 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 (since 2001)

to "gradually" reduce the levels and exposure

- Inventory of existing levels
  - First dioxins
  - Later dioxin-like PCBs: sum TEQ, ML dioxins kept
  - Limit around 90-95th percentile; so 5-10% above limit
- Also action limits (2/3 of limit)
  - For dioxins and for dl-PCBs



“Strict but feasible”

- Kind of ALARA but “reversed”
  - ALARA: as low as reasonable achievable
  - MLs may be lower than required for protection
  - Eg based on GAP in case of pesticides
- Some confusion: witch hunt on dioxins and PCBs

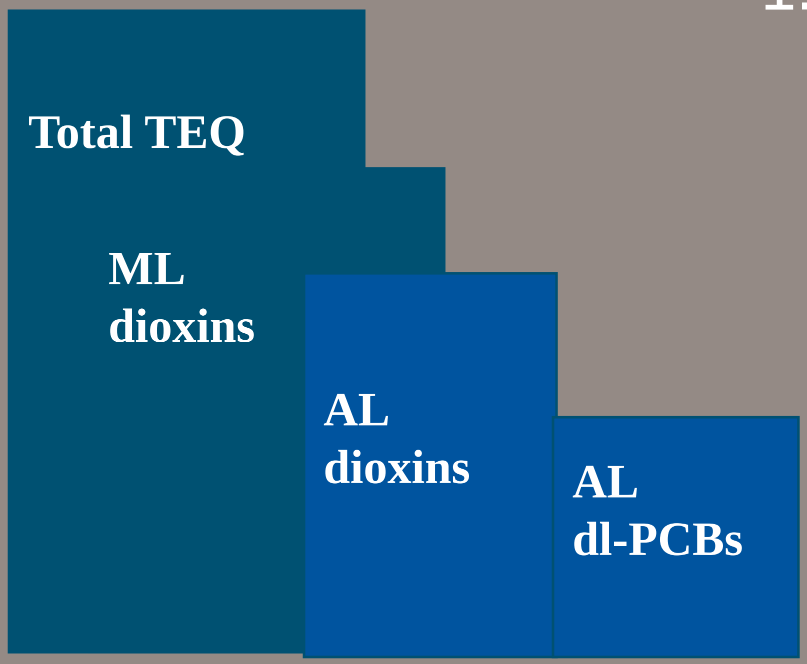
MLs protective?

- Not necessarily
- Example:
  - TWI: 14 pg TEQ/kg bw/week
  - ML fish dioxins and dl-PCBs: 6.5 pg TEQ/g fish
  - Recommended intake: 300 g/week (2 portions)
  - Intake: 1950 pg TEQ, about 30 pg TEQ/kg bw/week
  - So 2-fold higher than TWI
- In practice lower but fish is important source
- Similar applies for other food products
- So, products just below the MLs not necessarily safe,
  - in the long-term

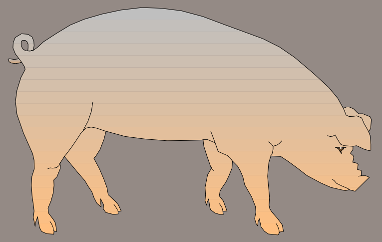
Food limits (since July 2002)

- Many different limits
  - 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
- New limits in 2012: change to “new” TEFs 2005
  - Food: Regulation (EU) No 1259/2011 amending Regulation (EC) No 1881/2006
  - Feed: Regulation (EU) No 277/2012 amending Annexes I and II to Directive 2002/32/EC

Action (AL) and Maximum (ML) levels



1.25 pg TEQ/g



1.0 pg TEQ/g

0.75 pg TEQ/g

0.5 pg TEQ/g

(EC) 1881/2006 and 2011/516/EU

Maximum and action levels

- If higher than maximum levels
  - Not allowed to sell the products
  - Recall
  - Not allowed to dilute
- If higher than action levels
  - Further action required to find the source of the contamination
  - Follow-up still limited
- Measurement uncertainty must be applied
  - To avoid false-positive results (95% certainty)

Current situation

Most products well below limits (by definition), except:

- part of the wild eel
- some free-range eggs
  - Due to soil consumption by hens
  - Still various farm-related incidents
- during incidents
  - Reported on RASFF system (not exclusive)
  - http://ec.europa.eu/food/food/rapidalert/index_en.htm

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Developments in levels

- Levels in human blood/milk declined in past decades
  - Up to 100 pg TEQ/g fat in seventies
  - Now around 10 pg TEQ/g fat
- Levels in food and feed expected to further decline
  - Thus far no clear declining trends in feed and food levels during last 10 years (EFSA 2010, 2012)
  - Issue: suitability data for trend analysis
- Much more (self) control
  - More sources will be detected and possibly removed

Questions?