

Risks of dioxins and dioxin-like PCBs

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Adverse health effects of dioxins



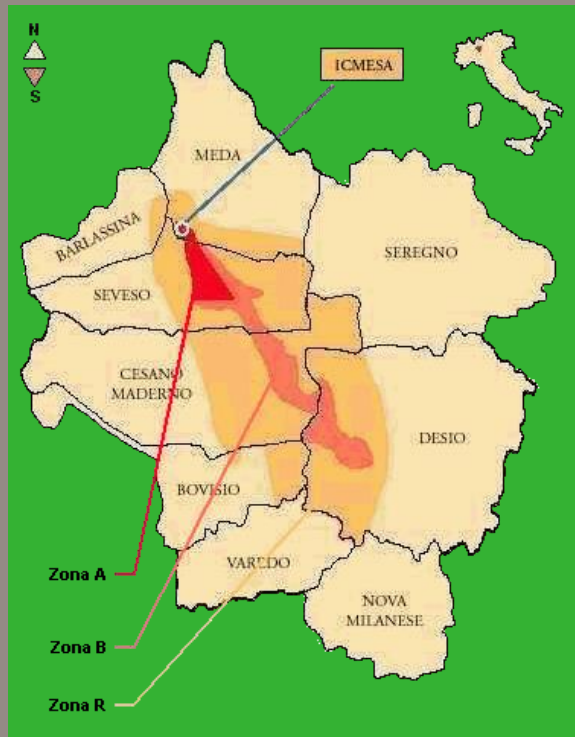
Victor Yushchenko: poisoned with a few mg of TCDD (2004)

Seveso 1976



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

Seveso, first effects



Zone	Subjects	Chloracne	Percent
	3-14 Yrs.	cases	
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 2000-56000 pg TEQ/g fat
 - Normally around 20 pg TEQ/g fat, so 100-2800x lower)
- Follow-up studies (still ongoing):
 - Increased incidence soft tissue sarcomas, haemopoietic neoplasms, liver and breast cancer
 - Decreased sperm counts in boys exposed at infancy and breast feeding
 - Increased incidence Diabetes mellitus
 - Change of sex ratio (more girls)
- But, 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."



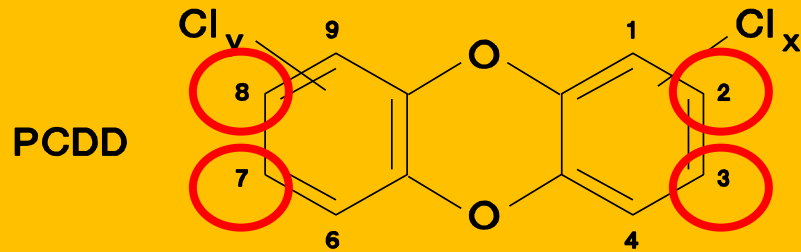
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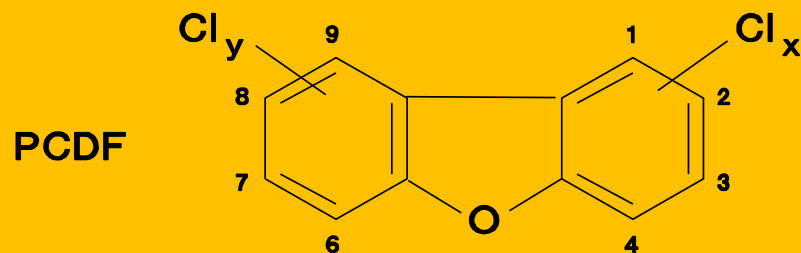
Dioxins and PCBs

- Which compounds ?
- Effects
- Risk assessment TCDD
- TEQ principle
- Incidents

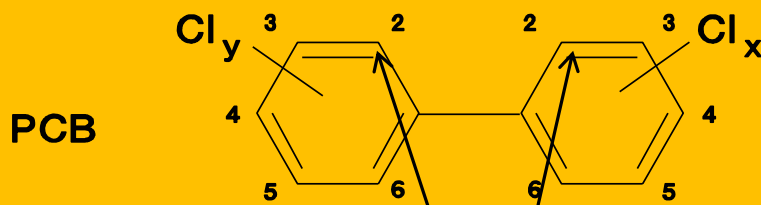
Dioxins (PCDD/Fs) and dioxin-like PCBs



7 out of 75



10 out of 135



12 out of 209

ortho

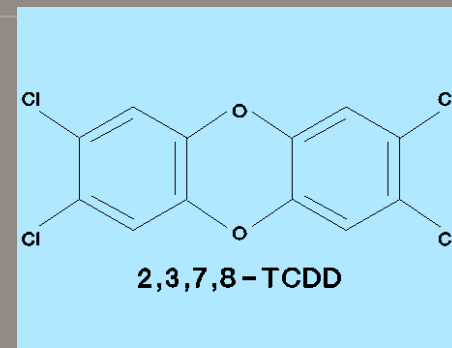
Properties of dioxins and planar PCBs

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



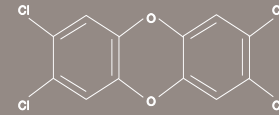
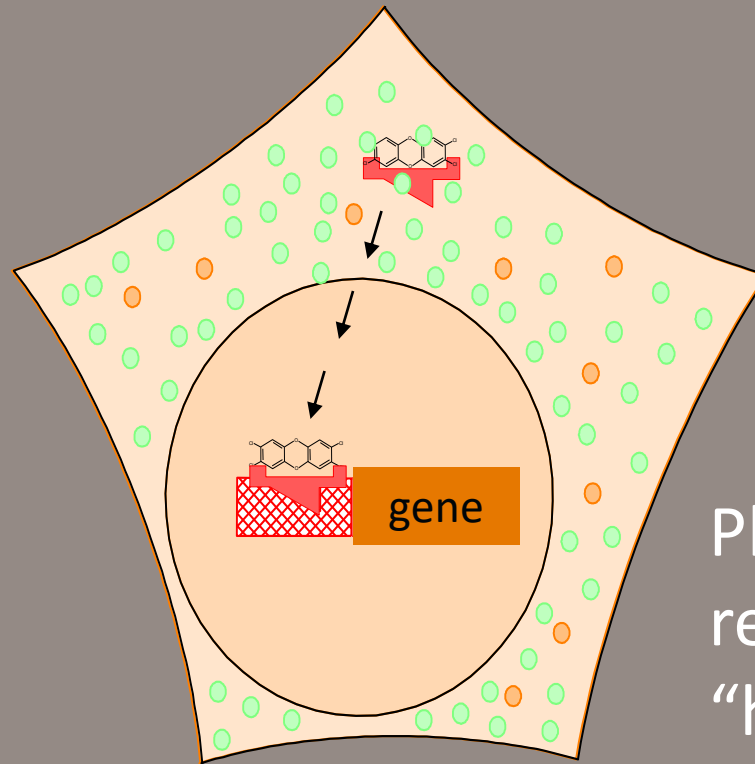
Toxicity of TCDD

(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 (Ah)-receptor



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

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

Note. Values are means \pm 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

Toxicity of TCDD: hazard characterization

Safe body burden

- 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.

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
- TDI of 1-4 pg TEQ/kg b.w./day with factor of 10 (WHO)

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.

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

JECFA (TMI)

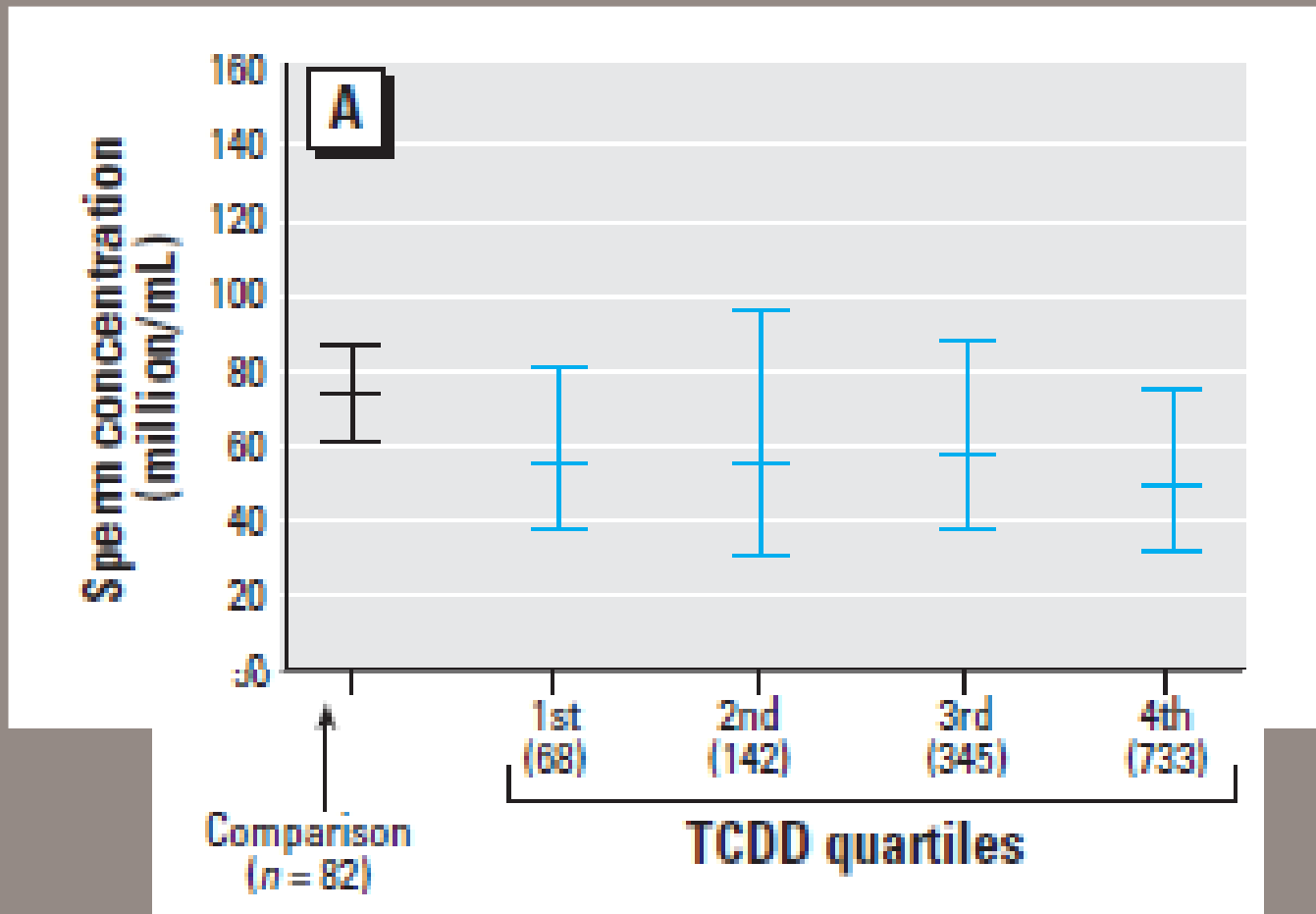
- JECFA: TMI of 70 pg TEQ/kg b.w./month
- Further extension possible, e.g. TYI?
 - Not necessarily, single high dose (incident!) may give higher exposure of e.g. foetus
 - So depends on effect intake on blood levels
- So: exposure is chronic, but effects may be (more) acute



Exposure limit US-EPA (Reference dose)

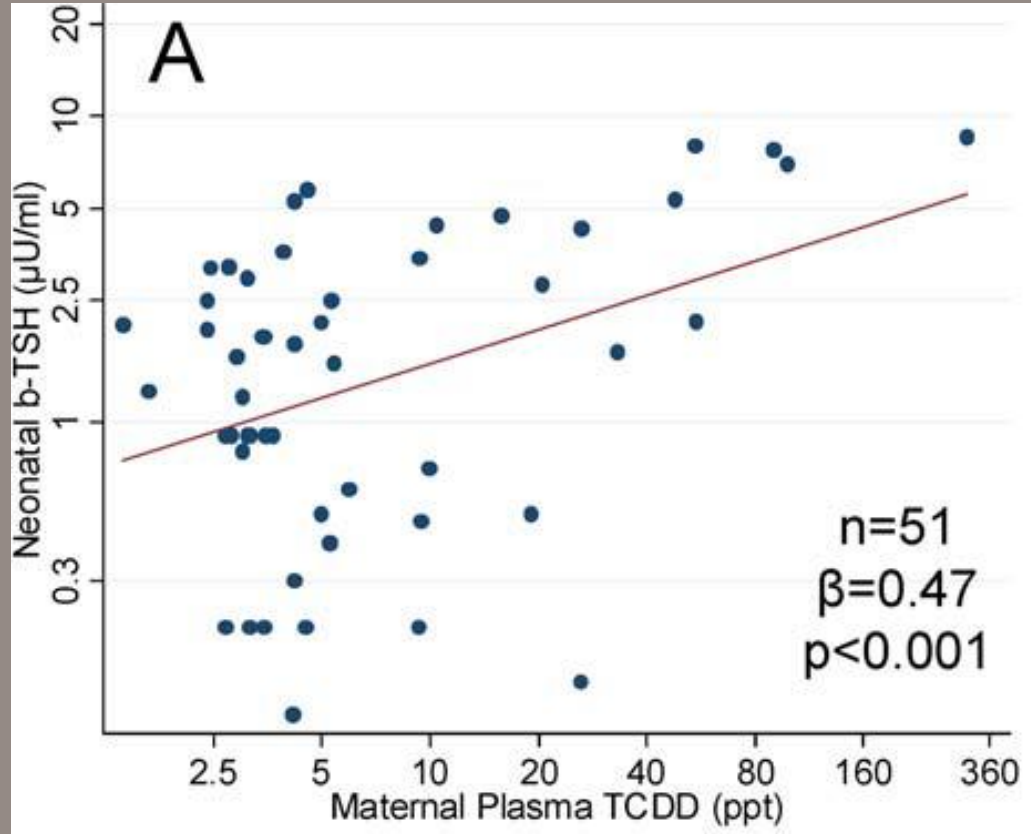
- US-EPA (2012): RfD of 0.7 pg TEQ/kg bw/day
- Based on new human studies from Seveso
 - Effects on sperm production in men exposed as young boys (Mocarelli et al. 2008)
 - Effects on thyroid hormones in children exposed in utero (Baccarelli et al. 2008)
- Use of human PBK-model (Emonds)
- Uncertainty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

Mocarelli et al. 2008



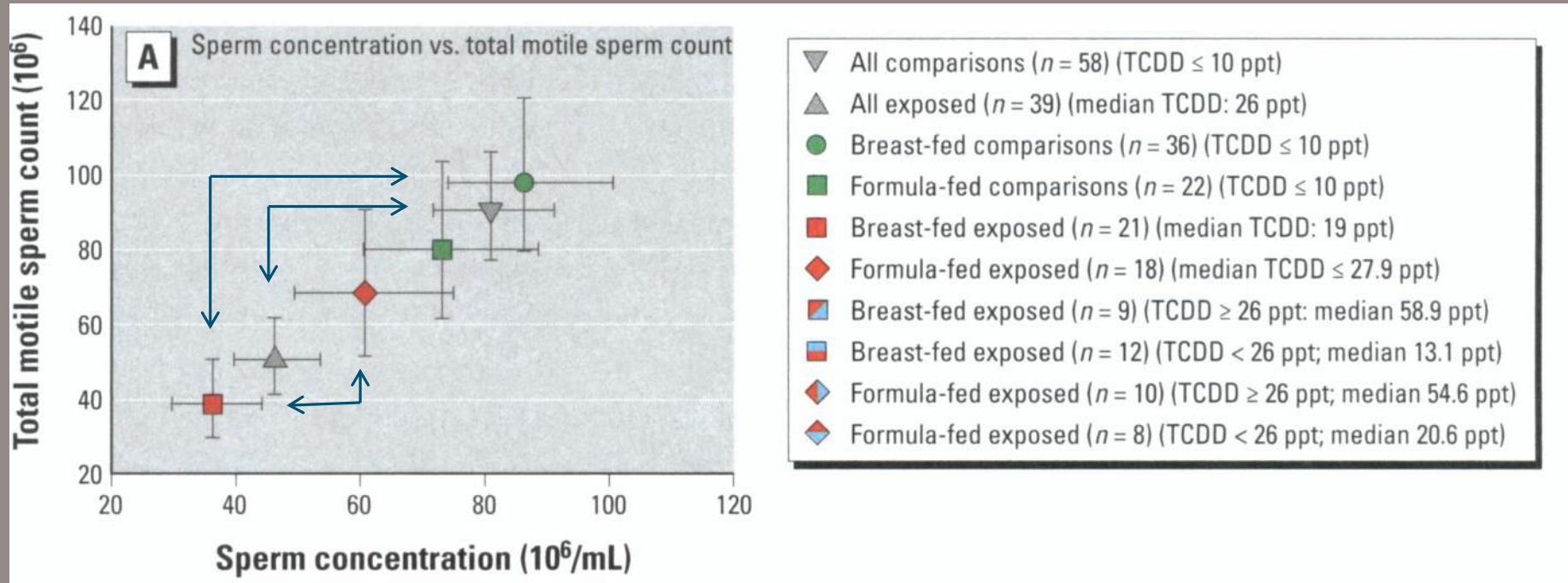
Man exposed at young age (background also high)

Baccarelli et al. 2008: TSH levels



Increased TSH levels in babies

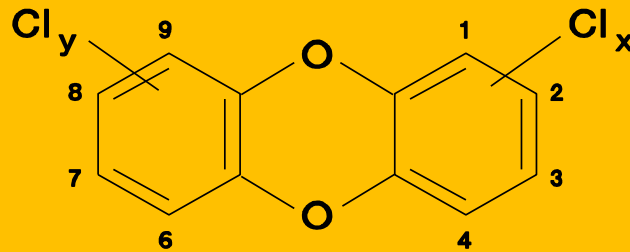
Mocarelli et al. 2011: perinatal exposure



- Clear effect on breast fed children
- Not yet used for risk assessments

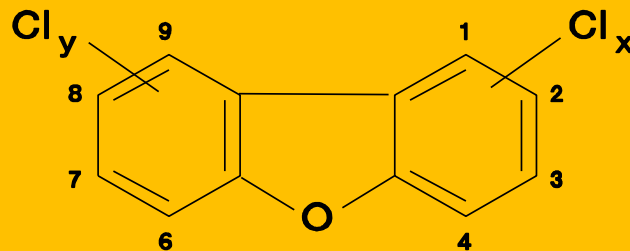
Dealing with mixtures

PCDD



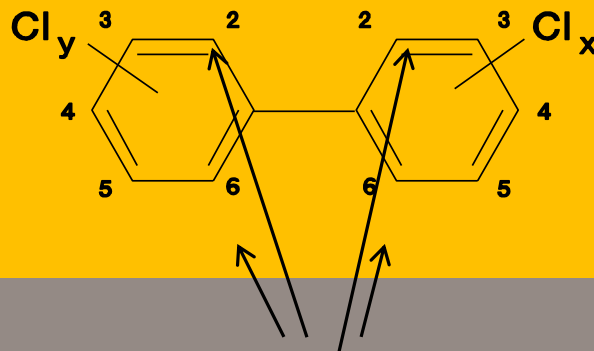
7 out of 75

PCDF



10 out of 135

PCB



12 out of 209

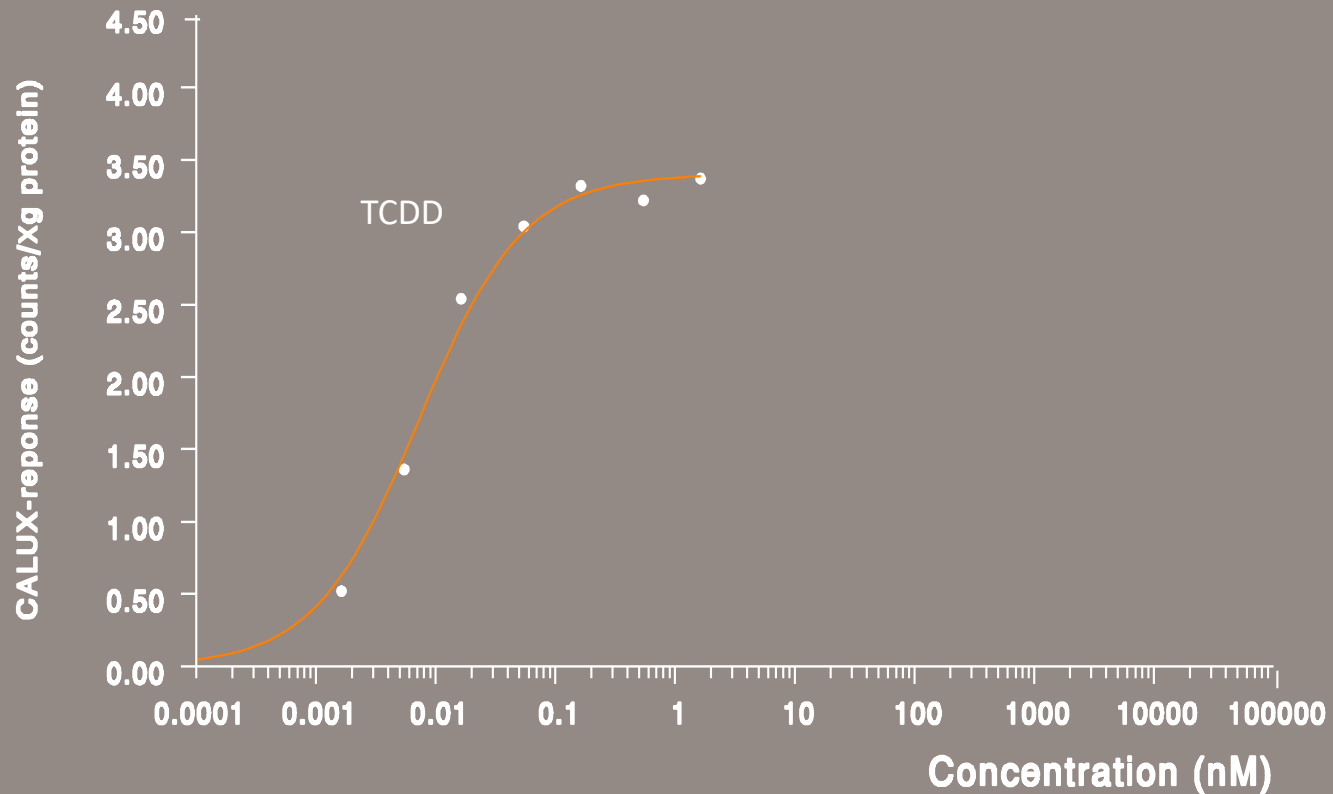
ortho



TEF values (Toxic Equivalency Factors)

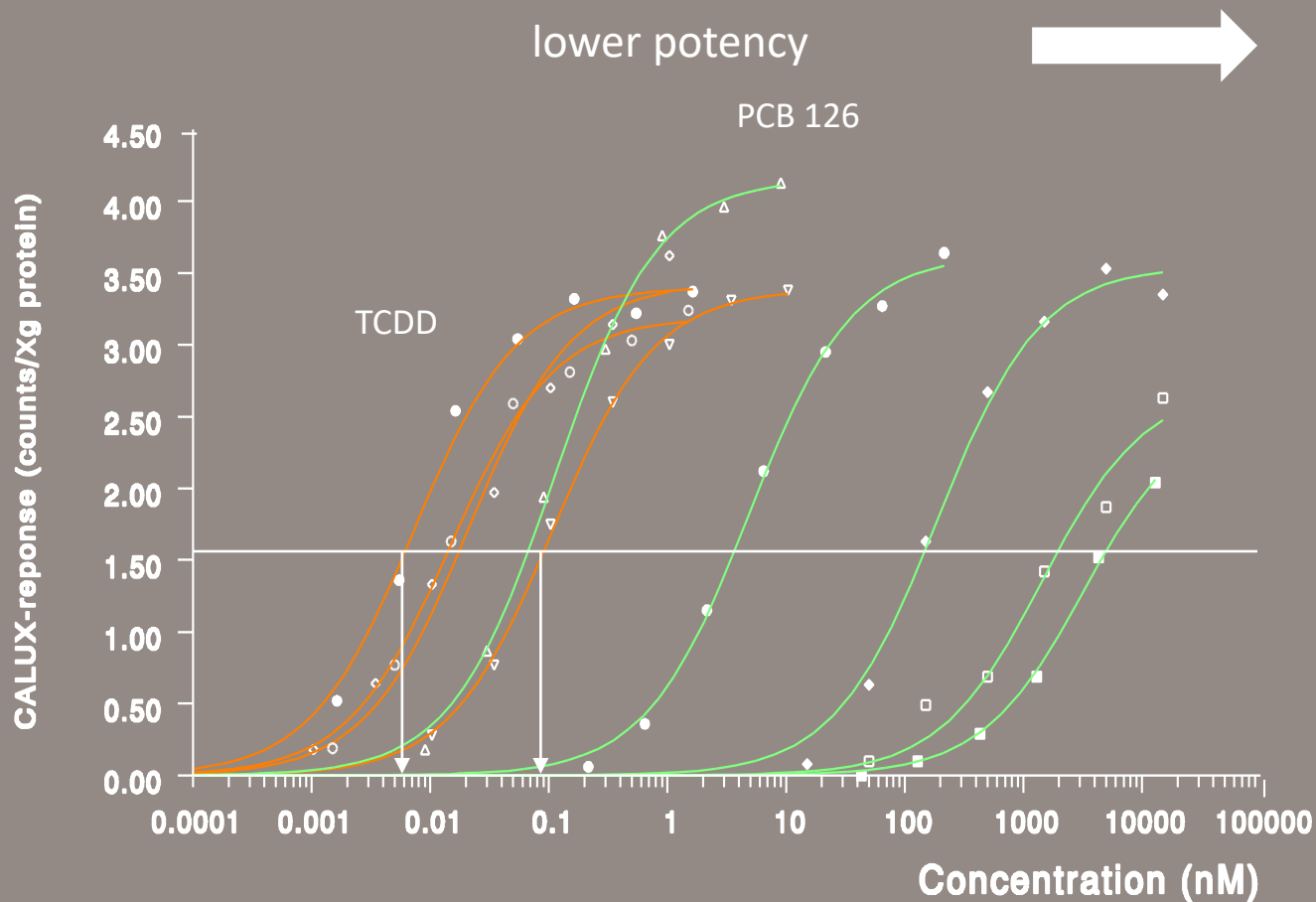
- How to deal with this **mixture** of congeners with different toxic potencies?
- Exposure limits (health based guidance values) apply for sum of dioxins and dioxin-like PCBs
- TEQ-principle:
 - Estimate the toxic potency of every dioxin and dioxin-like PCB in comparison to TCDD
 - TEF TCDD set at 1

Dose-response curves for dioxins and dl-PCBs



Bovee et al. 1998

Dose-response curves for dioxins and dl-PCBs



TEQ-principle

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

Establishment TEFs

- Broad range of values: TEF is weighted value
- Level of mixture expressed in TEQ:
 - $TEQ = \sum (\text{congener}_i \text{ level}) \times TEF_i$
- Last revised in 2005, but only since 2012 applied for official control in EU: check against product limits
- Last revision
 - Lower TEFs for mo-PCBs
 - Set on log-scale, so 1, 0.3, 0.1, 0.03 etc.
- Change of TEFs has effect on TEQ levels
 - Last change: about 15% reduction in levels

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



Calculation TEQ level: Belgian feed 1999

congener	WHO	Level	
	TEF (1998)	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 '99

congener	WHO	Level	
	TEF (2005)	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%)



Change in TEFs required?

- For most congeners no real change expected
- 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
 - Reduction would have huge impact on TEQ levels
 - Data enough to reduce the TEF? How to study in humans?

Incidents in the food chain



Dioxins in the news

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



Source: The Sydney Morning Herald



AllAboutFeed.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

1957: chickens discover dioxins



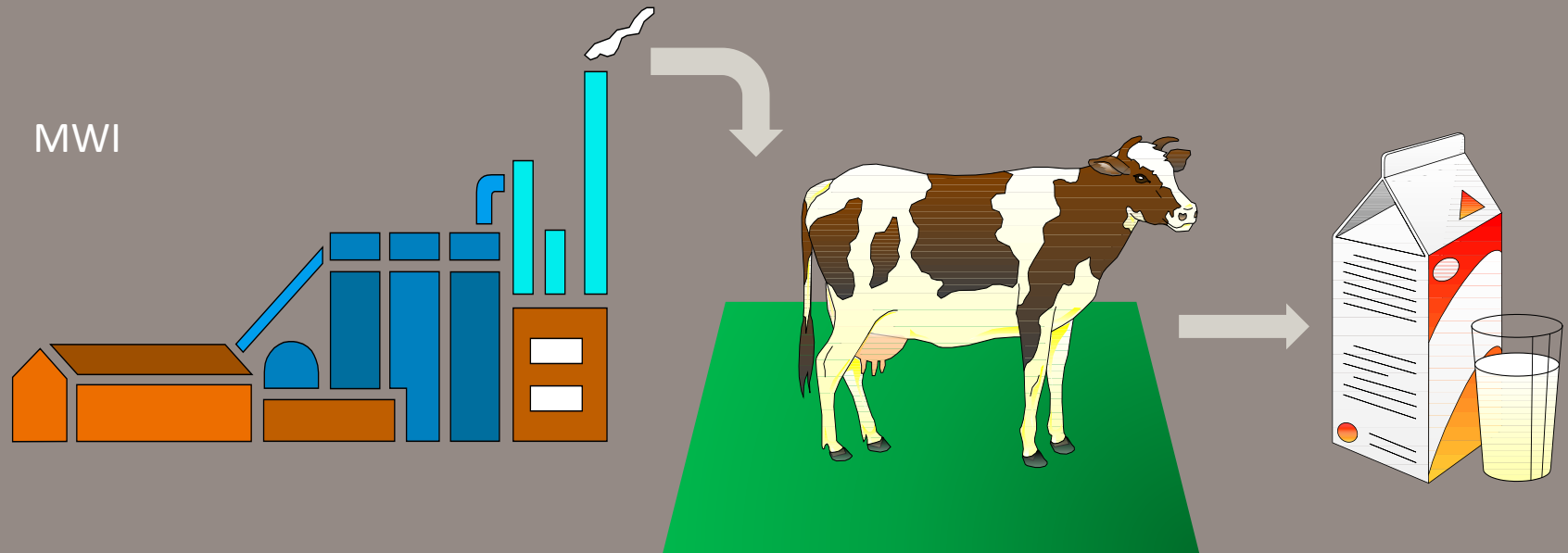
- Millions of dead and diseased chickens in US
- Chicken oedema disease
- After ten years dioxins identified as toxic agent
- Source: fat scrapings from cow hides that were treated with polychlorophenols
- Another chicken incident in 1969 in North Carolina due to wastewater from pesticide plant, with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

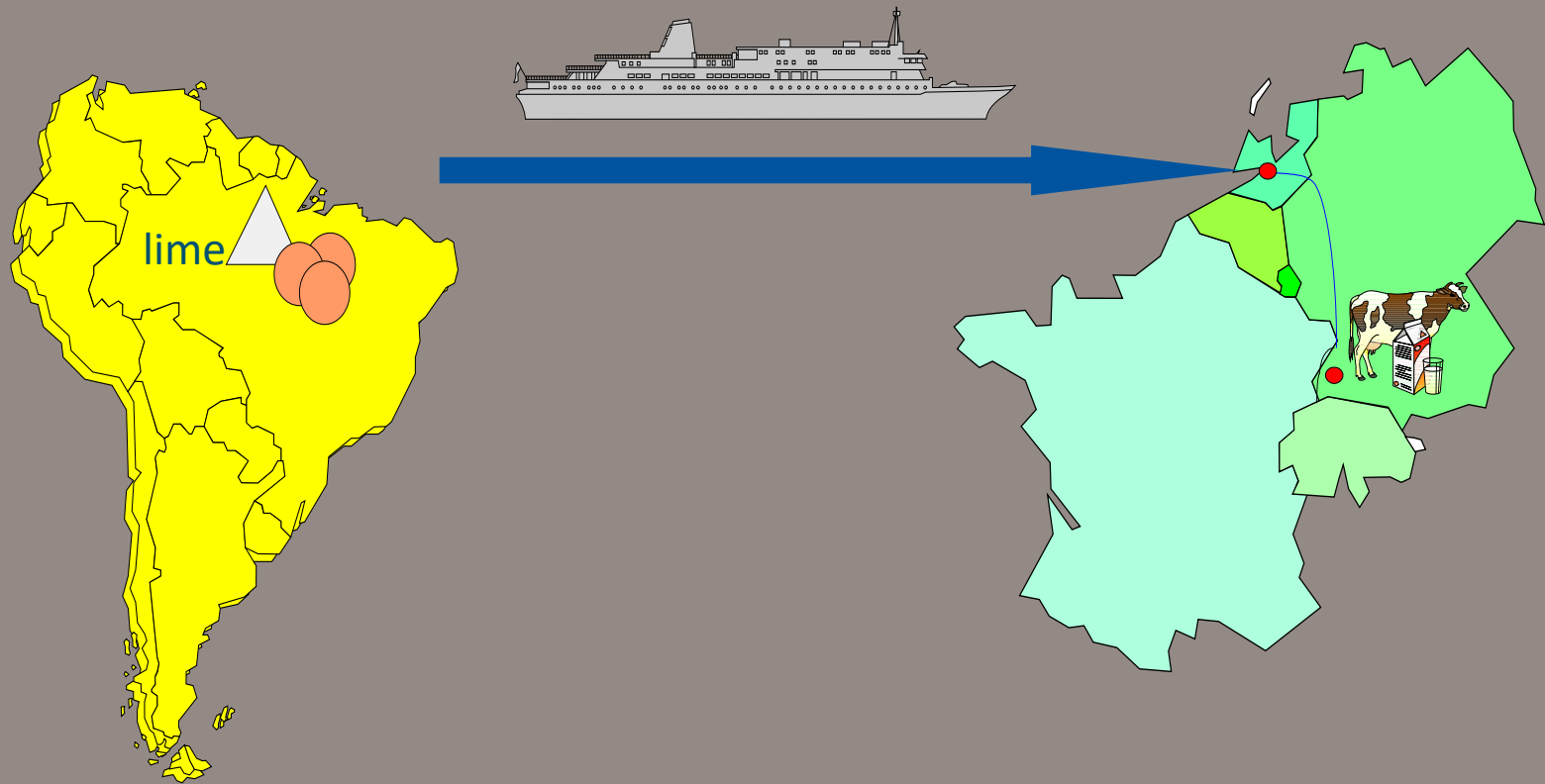


Dioxins in Dutch milk: waste incineration (1989)



- 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)



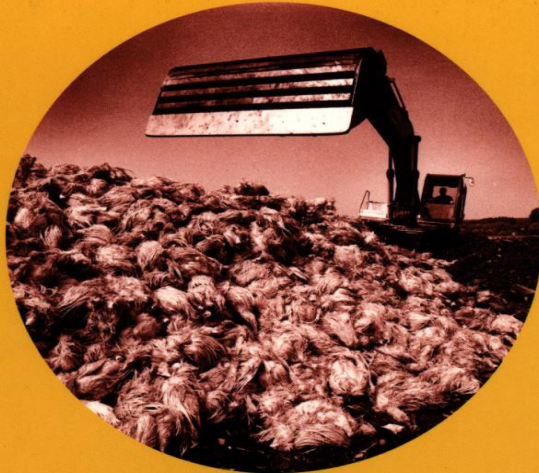
Use of contaminated lime for lowering water content and pH increase



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The Belgian dioxin crisis in 1999



**Vet drijft
altijd boven**

SIEL VAN DER DONCKT

*De verzwegen
dioxinecrisis*

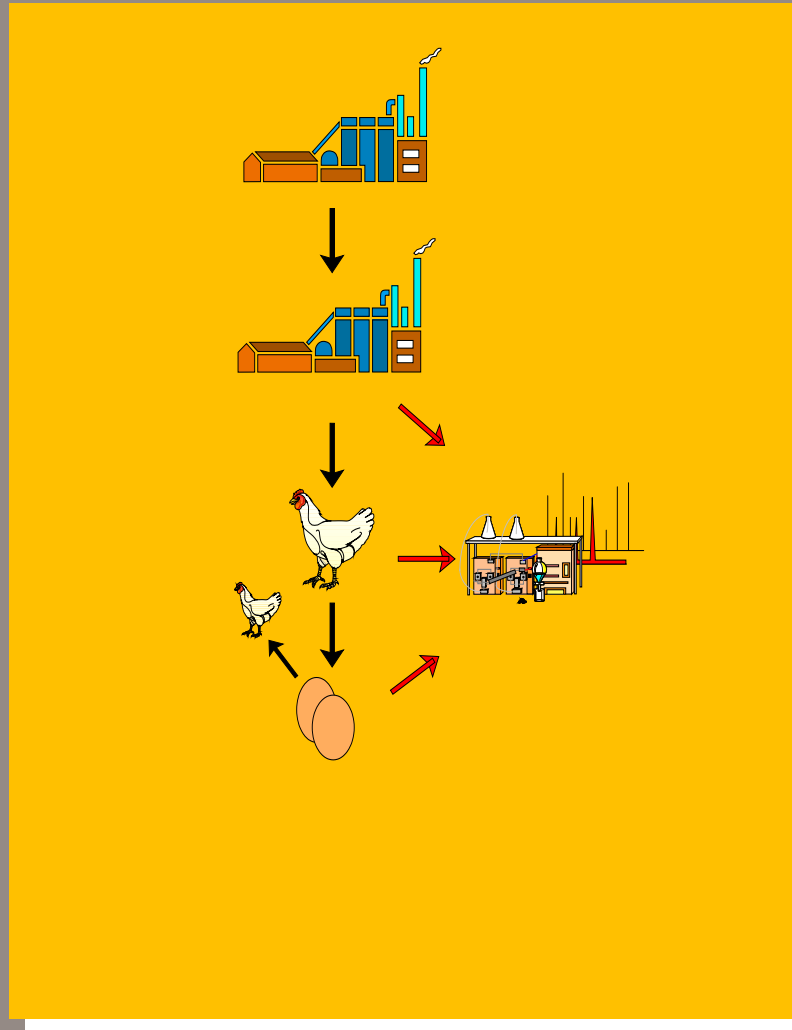


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Dioxins again discovered by chickens

- 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

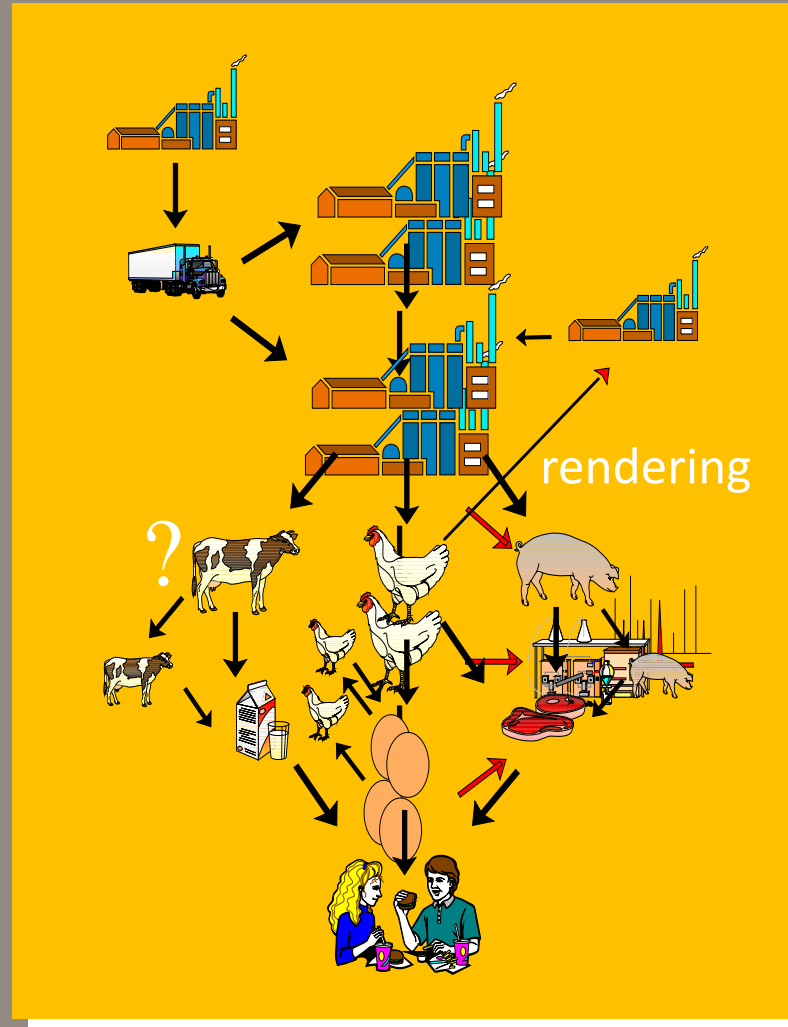
*Background levels below 5 pg WHO-TEQ/g fat

**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: 200 liter PCB-oil !

Development of the crisis



Testing of samples during the crisis

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

Consequences Belgian crisis

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

Other consequences Belgian dioxin crisis

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

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



Risk assessment by EFSA

- Only part of the meat contaminated
- Intake will lead to temporary intake above TWI
- Effect of occasional exposure on body burden is limited
- Good communication to the public
 - Contaminated farms rapidly traced and blocked
 - Many food products taken from shelves

Calculation

- 150 gram pork, 10% fat 600 pg TEQ/g fat
 - $15 \times 600 \text{ pg TEQ} = 9000 \text{ pg TEQ}$
 - Or $9000/60 \text{ kg} = 150 \text{ pg TEQ/kg bw}$
 - About 10x TWI
- Compared to existing body burden of 150-300 ng TEQ
 - 3-6% increase from 1 meal
- However, much higher levels in pork liver
 - 16.000 pg TEQ/g fat, 5% fat or 120 ng TEQ/150 g
 - Not taken into account
 - What is actual consumption of pork liver?

Contaminated eel



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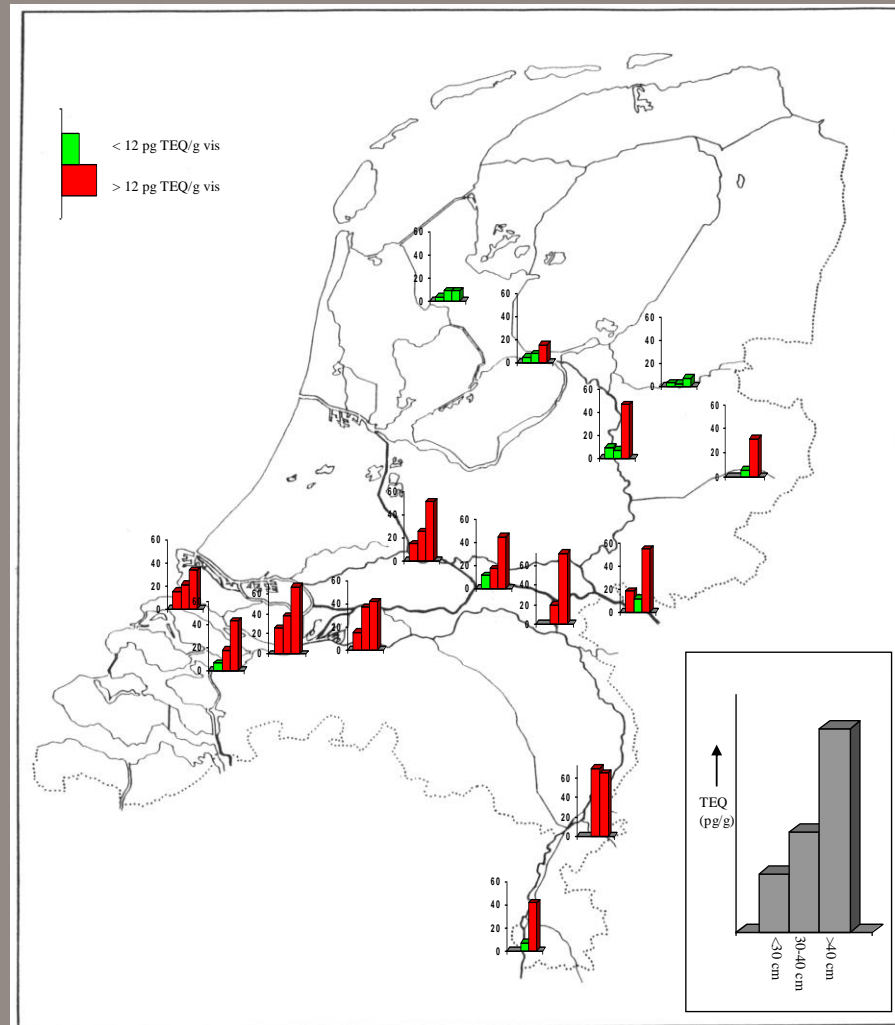
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Dioxins and dl-PCBs in wild eel

- In the Netherlands 200-300 tons of wild eel/year
- 5-10% of total eel production, rest farmed eel



Locations and levels



Levels according to length

Length (cm)	Fat content (%)	Sum dioxins and dl-PCBs		
		mean	(range)	positive (%)
30-40	11	27.0	(2.0-70.5)	68
>40	21	48.1	(6.7-74.5)	91

Limit 12 pg TEQ/g



Risk of wild eel for consumer

- Based on consumption of only wild eel
 - In practice most eel is farmed eel, but “preference” for wild eel cannot be excluded
- Consumption **one portion per month of 150 grams**
- Average level Biesbosch eel **29 pg TEQ/g** (TEFs₂₀₀₆)
- Intake per portion 4.3 ng TEQ or 66 pg TEQ/kg bw
 - Overall estimation **79 pg TEQ/kg bw** due to higher background exposure fish eaters
- Thus about 5x TWI (14 pg TEQ/kg bw/week), but consumption only once a month

■ So what?



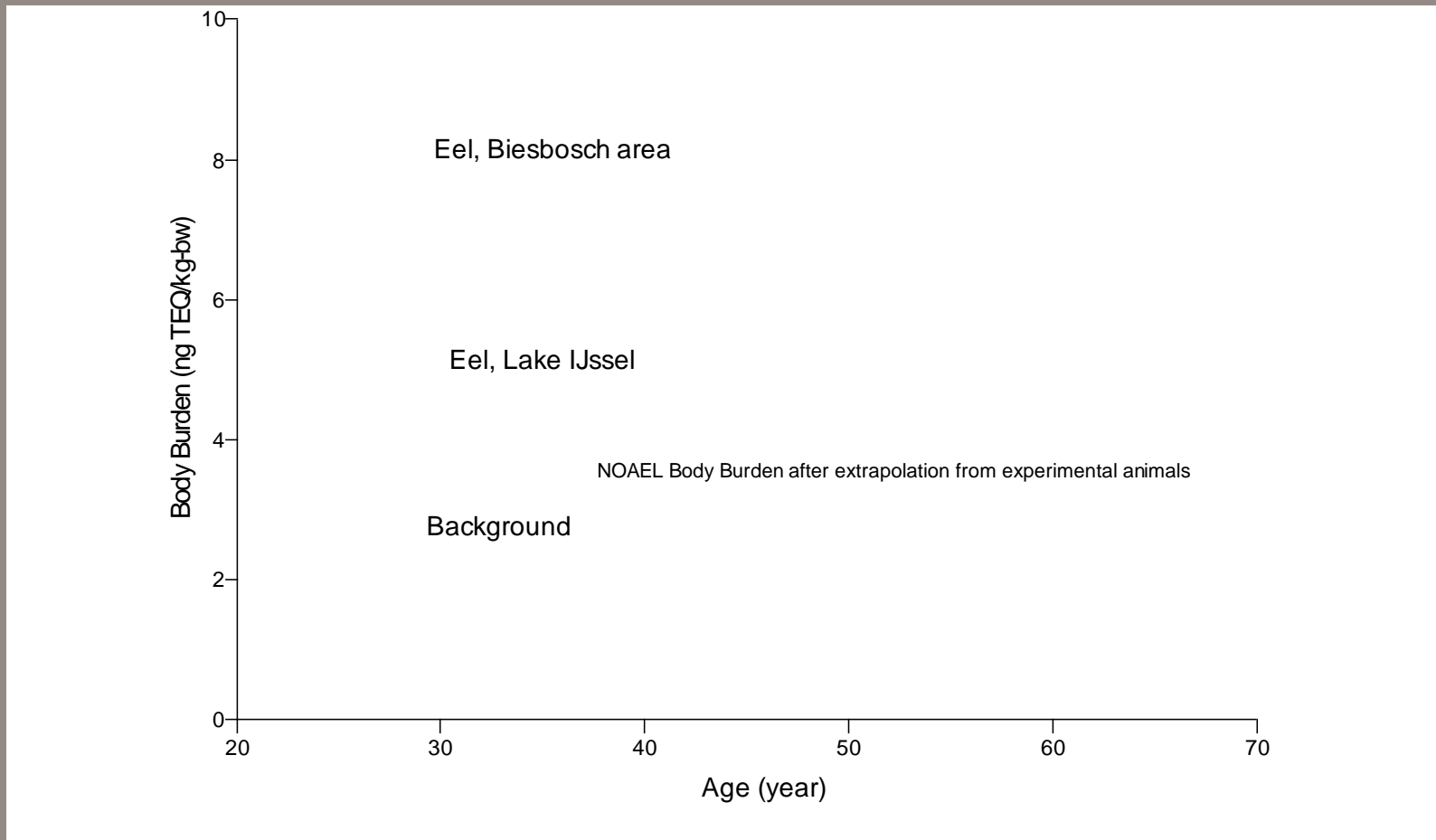
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Estimated effect on body burden

- Consumption of only Biesbosch eel or Lake IJssel eel, once a month 150 grams
 - Biesbosch eel: 29 pg TEQ/g eel
 - Lake IJssel eel: 7 pg TEQ/g eel
- Starting point 20 years of age
- Start body burden estimated from mothers milk; 95th percentile since fish eaters have higher intake
- Compared to **NOAEL body burden** based on effects on sperm count in young rats, with UF of 10: **4 ng TEQ/kg bw**

Effect eel consumption on body burden



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Questions?



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