Transfer of dioxins and dl-PCBs in farm animals

Ron Hoogenboom

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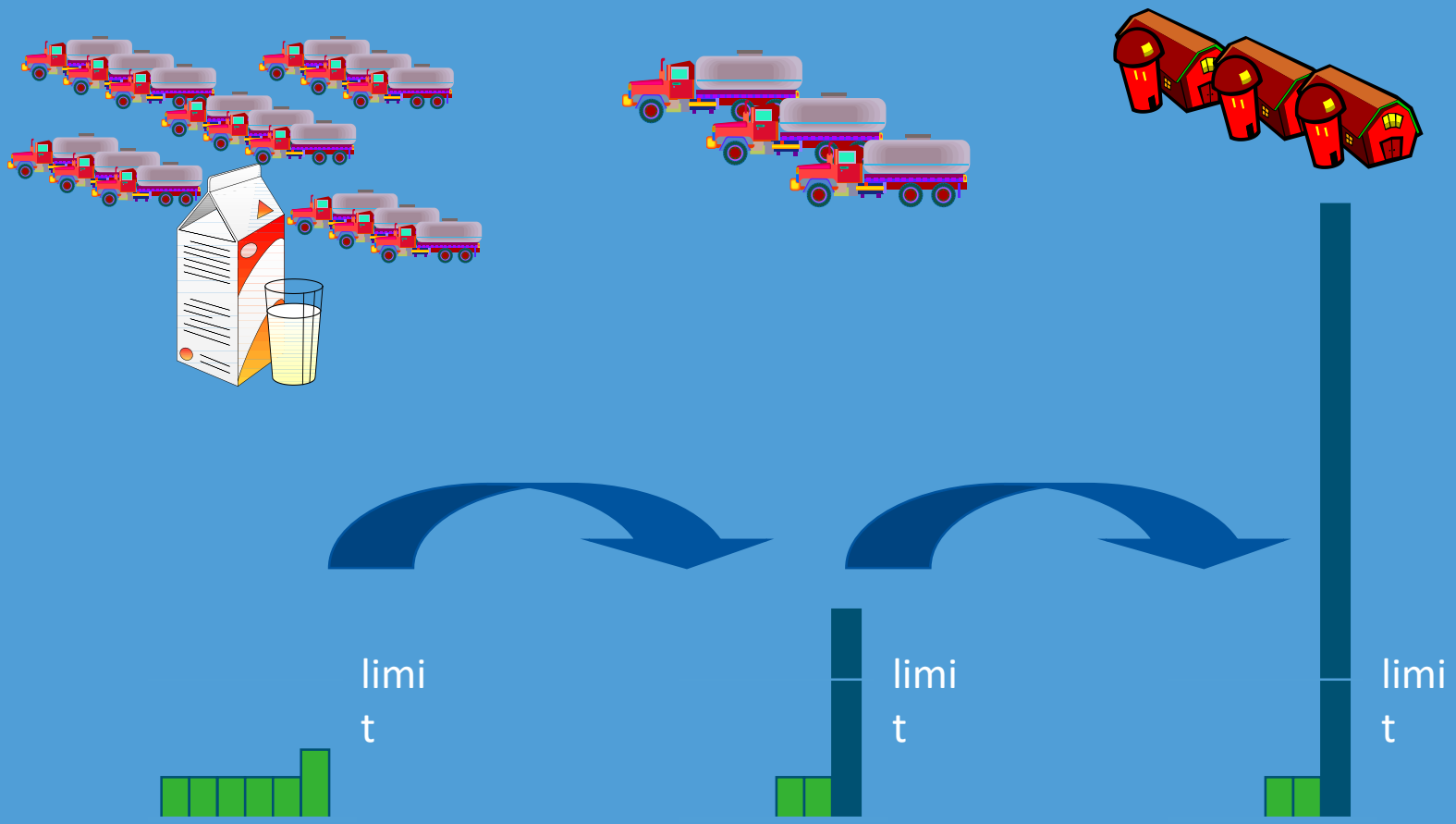
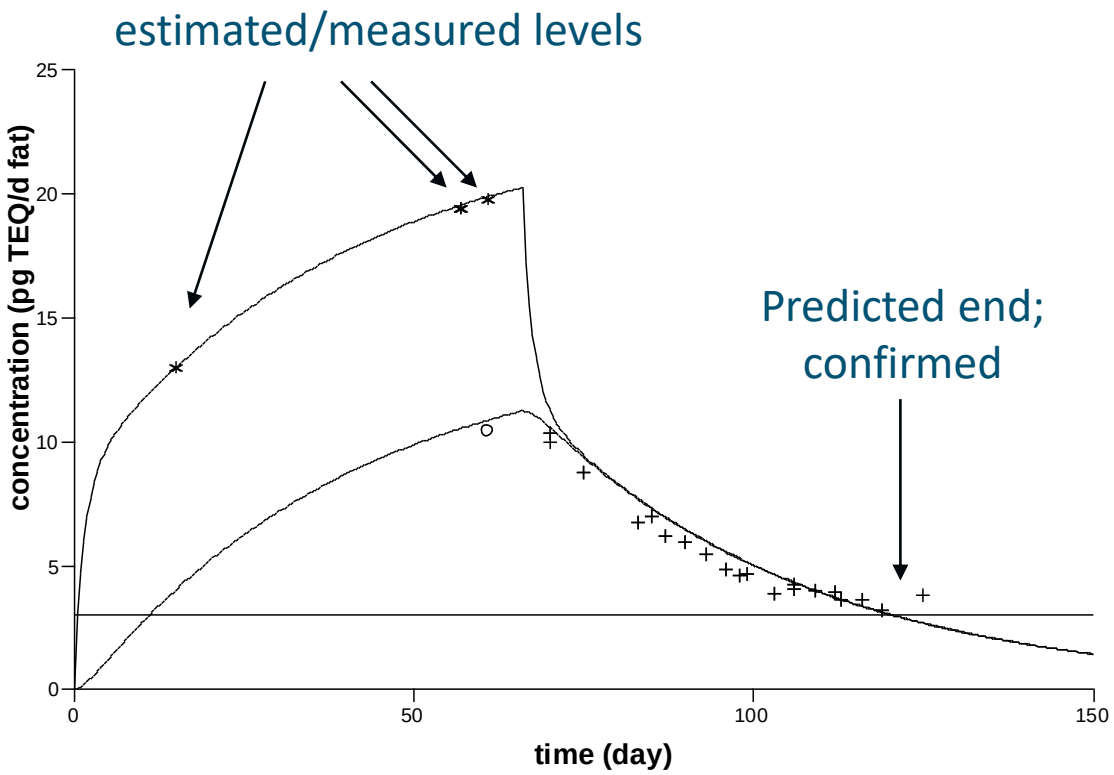
Clay incident 2004: discovery and T&T



Major questions

- Where do the dioxins come from?
    - How widespread is this?
- When did this start?
- When will the milk have levels below the limit?

Application of PBPK model by RIVM



Studies on transfer

- Two types of studies
  - Follow-up of incidents
  - In this case only depletion kinetics
- Well-controlled animal studies
- Several studies performed in the Netherlands
  - Often related to incident
- Studies on milk and eggs give most information,
  - Animals don’t need to be killed
  - Laying hens are small

Dairy cows

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Transfer rates dairy cows

Table 2
Carry-over[a] rates in % as reported for lactating cows in various studies and the present study.

Daily dose (ng TEQ kg bw^{-1} day^{-1})	Slob 1995	Slob 1995	McLachlan and Richter, 1998		Malisch 2000	Huwe 2005	Brambilla 2008
	2.5	0.2	0.002	0.019	0.02	0.23	0.026
Source	Fly ash	MWI	Natural	Sludge	Citrus pulp	MgO	Minerals
Number of cows	2	Many	4	4	Many	2	1604
Duration exposure (d)	1	30	84	23	180	40	28
Withdrawal period (d)	56	–	–	–	350	40	
kg milk fat day^{-1}	1.0	1.0	0.9	1.0	1.2	0.7	1.0
Study design	Stable	Field	Stable	Stable	Field	Stable	Field
2,3,7,8-TCDD	0.9	15	38	51	58	nd	29.7
1,2,3,7,8-PeCDD	0.7	10	39	27	49	26.9	54.2
1,2,3,4,7,8-HxCDD	0.3	5.6	33	21	51	20.0	40.9
1,2,3,6,7,8-HxCDD	0.3	6.4	33	13	77	27.3	48.8
1,2,3,7,8,9-HxCDD	0.1	3.1	16	10	35	15.8	28.6
1,2,3,4,6,7,8-HpCDD	0.05	0.6	3.4	2.0	18	4.8	10.3
1,2,3,4,6,7,8,9-OCDD	0.03	0.1	0.7	0.3	3.7	0.7	0.4
2,3,7,8-TCDF	0.03		nd	nd	2.8	nd	–
1,2,3,7,8-PeCDF	0.02		nd	nd	3.8	2.3	4.2
2,3,4,7,8-PeCDF	0.8	12.0	40	65	58	23.8	55.6
1,2,3,4,7,8-HxCDF	0.3	4.3	24	23	33	21.3	42.4
1,2,3,6,7,8-HxCDF	0.2	3.6	19	27	30	20.6	43.9
2,3,4,6,7,8-HxCDF	0.4	4.2	19	20	19	17.5	38.9
1,2,3,7,8,9-HxCDF	0.05				nd	nd	8.1
1,2,3,4,6,7,8-HpCDF	0.04	0.4	3.4	1.9	3.1	4.4	10.2
1,2,3,4,7,8,9-HpCDF	0.05	0.5	nd	3.6	4.2	5.6	11.0
1,2,3,4,6,7,8,9-OCDF	0.02			0.3	0.4	0.5	0.4
PCB77		1.2					
PCB81							
PCB126		35.0				26.1	
PCB169		31.0				37.6	

[a] Carry-over rates are estimated from the intake through the feed and excretion in milk at "steady state".

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Impact of fires for agricultural activities

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Transfer rates dairy cows: what to use?

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1,2,3,6,7,8-HxCDD	0.3	6.4	33	13	77	27.3	48.8
1,2,3,7,8,9-HxCDD	0.1	3.1	16	10	35	15.8	28.6
1,2,3,4,6,7,8-HpCDD	0.05	0.6	3.4	2.0	18	4.8	10.3
1,2,3,4,6,7,8,9-OCDD	0.03	0.1	0.7	0.3	3.7	0.7	0.4
2,3,7,8-TCDF	0.03		nd	nd	2.8	nd	–
1,2,3,7,8-PeCDF	0.02		nd	nd	3.8	2.3	4.2
2,3,4,7,8-PeCDF	0.8	12.0	40	65	58	23.8	55.6
1,2,3,4,7,8-HxCDF	0.3	4.3	24	23	33	21.3	42.4
1,2,3,6,7,8-HxCDF	0.2	3.6	19	27	30	20.6	43.9
2,3,4,6,7,8-HxCDF	0.4	4.2	19	20	19	17.5	38.9
1,2,3,7,8,9-HxCDF	0.05				nd	nd	8.1
1,2,3,4,6,7,8-HpCDF	0.04	0.4	3.4	1.9	3.1	4.4	10.2
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PCB77		1.2					
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[a] Carry-over rates are estimated from the intake through the feed and excretion in milk at "steady state".

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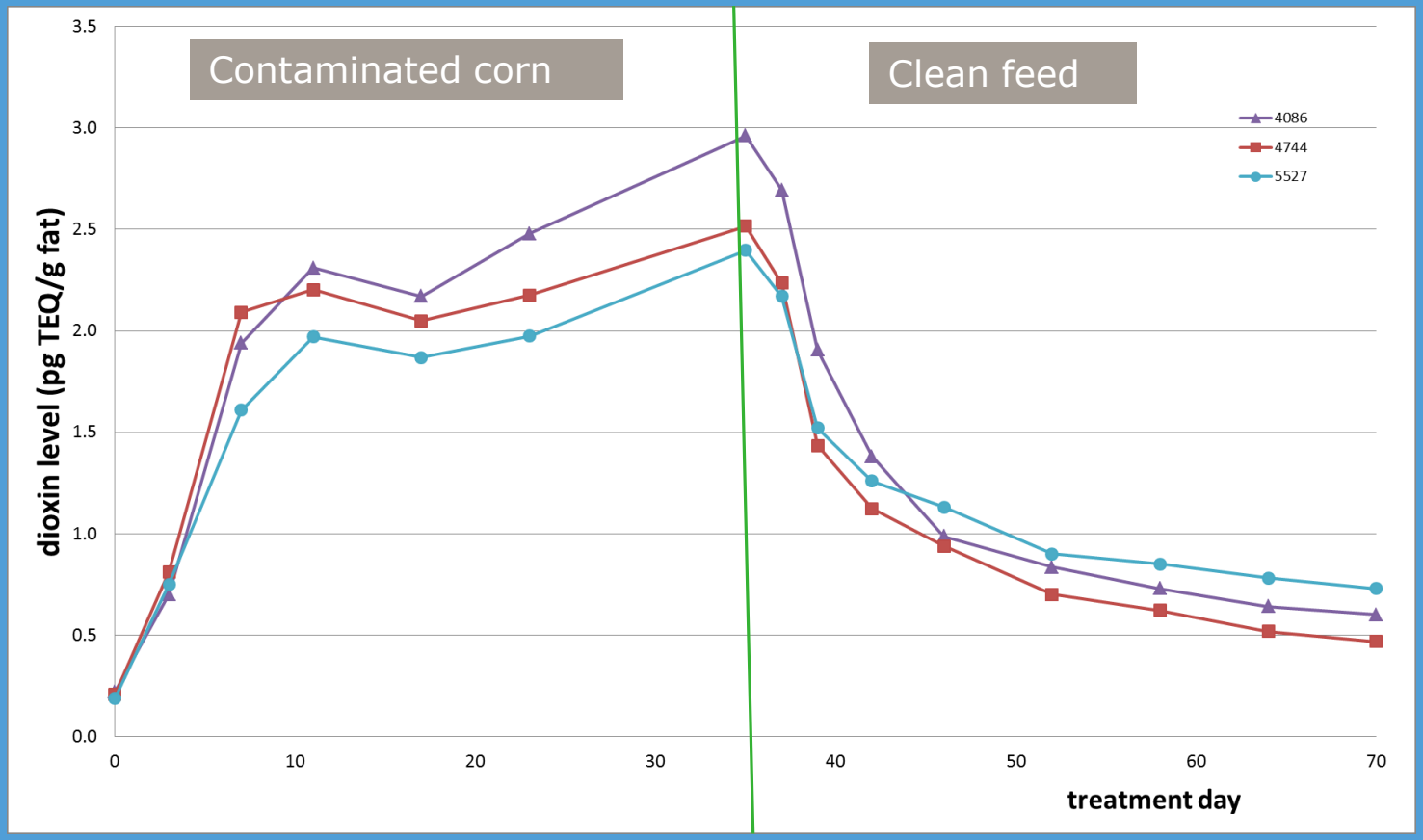
Carry-over of dioxins from contaminated corn in dairy cows

- Corn contaminated by smoke from PVC fire
  - Level of 0.8 ng TEQ/kg (ML 0.75)
- Study with 3 dairy cows
  - 5 weeks exposure
  - 5 weeks clean feed

Study dairy cows with contaminated corn silage

primarily dioxins due to fire



ML

AL

Cows fed 15 kg (dm) corn silage/day, 0.8 ng TEQ/kg (88% dm) for 5 weeks

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Transfer rates dairy cows

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	2.5	0.2	0.002	0.019	0.02	0.23	0.026	0.023	0.018
Source	Fly ash	MWI	Natural	Sludge	Citrus pulp	MgO	Minerals	Maize silage	Sugar beet
Number of cows	2	Many	4	4	Many	2	1604	3	3
Duration exposure (d)	1	30	84	23	180	40	28	33	33
Withdrawal period (d)	56	–	–	–	350	40			
kg milk fat day^{-1}	1.0	1.0	0.9	1.0	1.2	0.7	1.0	1.5	1.4
Study design	Stable	Field	Stable	Stable	Field	Stable	Field	Stable	Stable
2,3,7,8-TCDD	0.9	15	38	51	58	nd	29.7	30.3	36.1
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1,2,3,4,7,8-HxCDD	0.3	5.6	33	21	51	20.0	40.9	26.9	23.4
1,2,3,6,7,8-HxCDD	0.3	6.4	33	13	77	27.3	48.8	29.8	10.7
1,2,3,7,8,9-HxCDD	0.1	3.1	16	10	35	15.8	28.6	20.1	18.1
1,2,3,4,6,7,8-HpCDD	0.05	0.6	3.4	2.0	18	4.8	10.3	4.2	0.8
1,2,3,4,6,7,8,9-OCDD	0.03	0.1	0.7	0.3	3.7	0.7	0.4	0.2	0.0
2,3,7,8-TCDF	0.03		nd	nd	2.8	nd	–	1.8	1.1
1,2,3,7,8-PeCDF	0.02		nd	nd	3.8	2.3	4.2	4.1	2.7
2,3,4,7,8-PeCDF	0.8	12.0	40	65	58	23.8	55.6	30.7	24.6
1,2,3,4,7,8-HxCDF	0.3	4.3	24	23	33	21.3	42.4	24.2	11.5
1,2,3,6,7,8-HxCDF	0.2	3.6	19	27	30	20.6	43.9	25.5	12.1
2,3,4,6,7,8-HxCDF	0.4	4.2	19	20	19	17.5	38.9	23.3	8.8
1,2,3,7,8,9-HxCDF	0.05				nd	nd	8.1	7.3	nd
1,2,3,4,6,7,8-HpCDF	0.04	0.4	3.4	1.9	3.1	4.4	10.2	4.8	1.4
1,2,3,4,7,8,9-HpCDF	0.05	0.5	nd	3.6	4.2	5.6	11.0	6.7	0.0
1,2,3,4,6,7,8,9-OCDF	0.02			0.3	0.4	0.5	0.4	0.0	0.0
PCB77		1.2						2.0	0.0
PCB81								12.9	9.7
PCB126		35.0				26.1		32.4	36.1
PCB169		31.0				37.6		35.8	35.8

[a] Carry-over rates are estimated from the intake through the feed and excretion in milk at "steady state".

Laying hens

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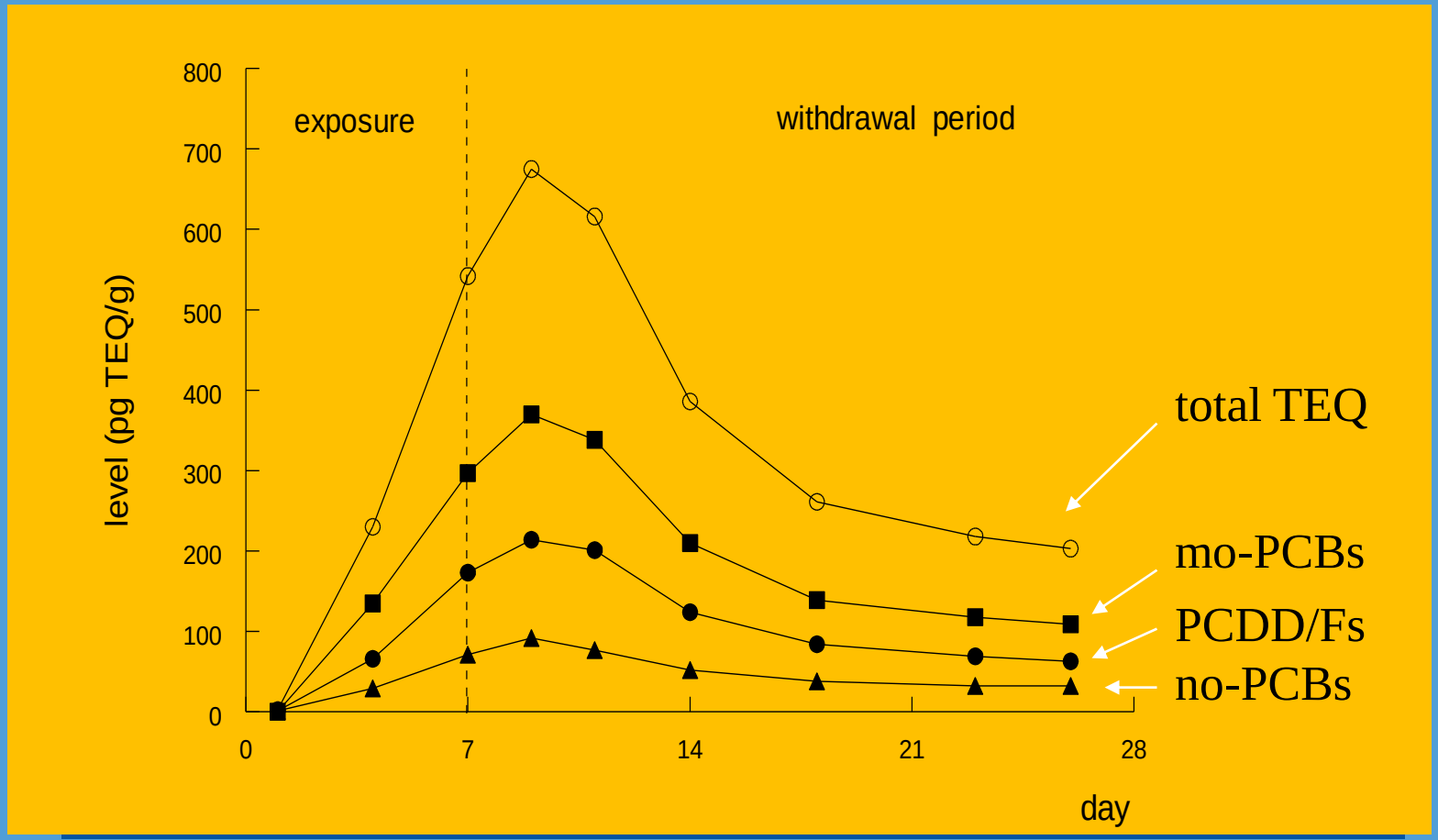
Study on dioxin and dl-PCB levels in eggs

Belgium feed 15x diluted; 1 week exposure, then clean feed



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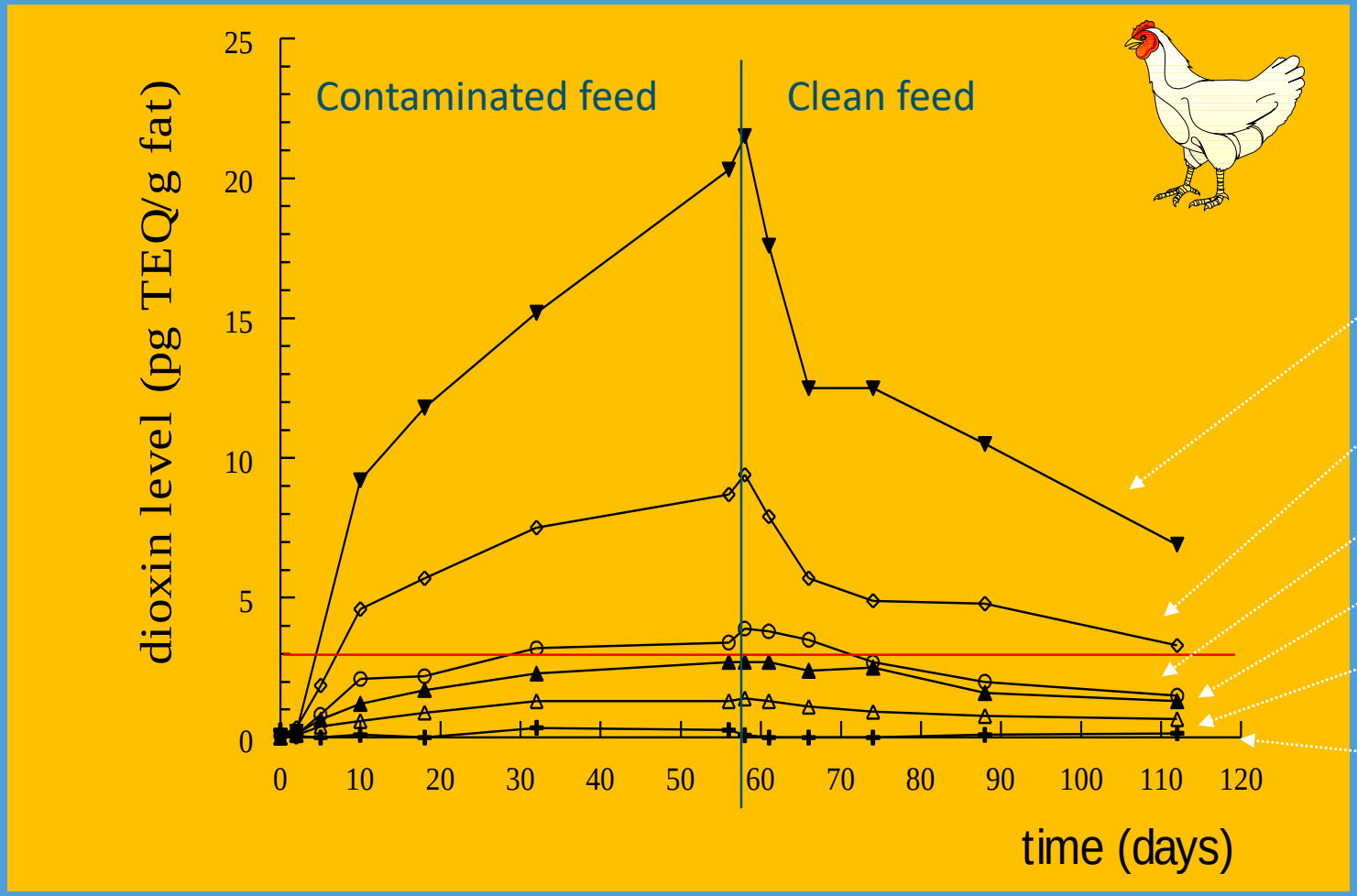
Follow-up study

- Mixture of dioxins and PCBs added to feed at different levels
    - Also feed with soil
- 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



Adapted from Hoogenboom et al. 2006, Chemosphere 68, 814-823

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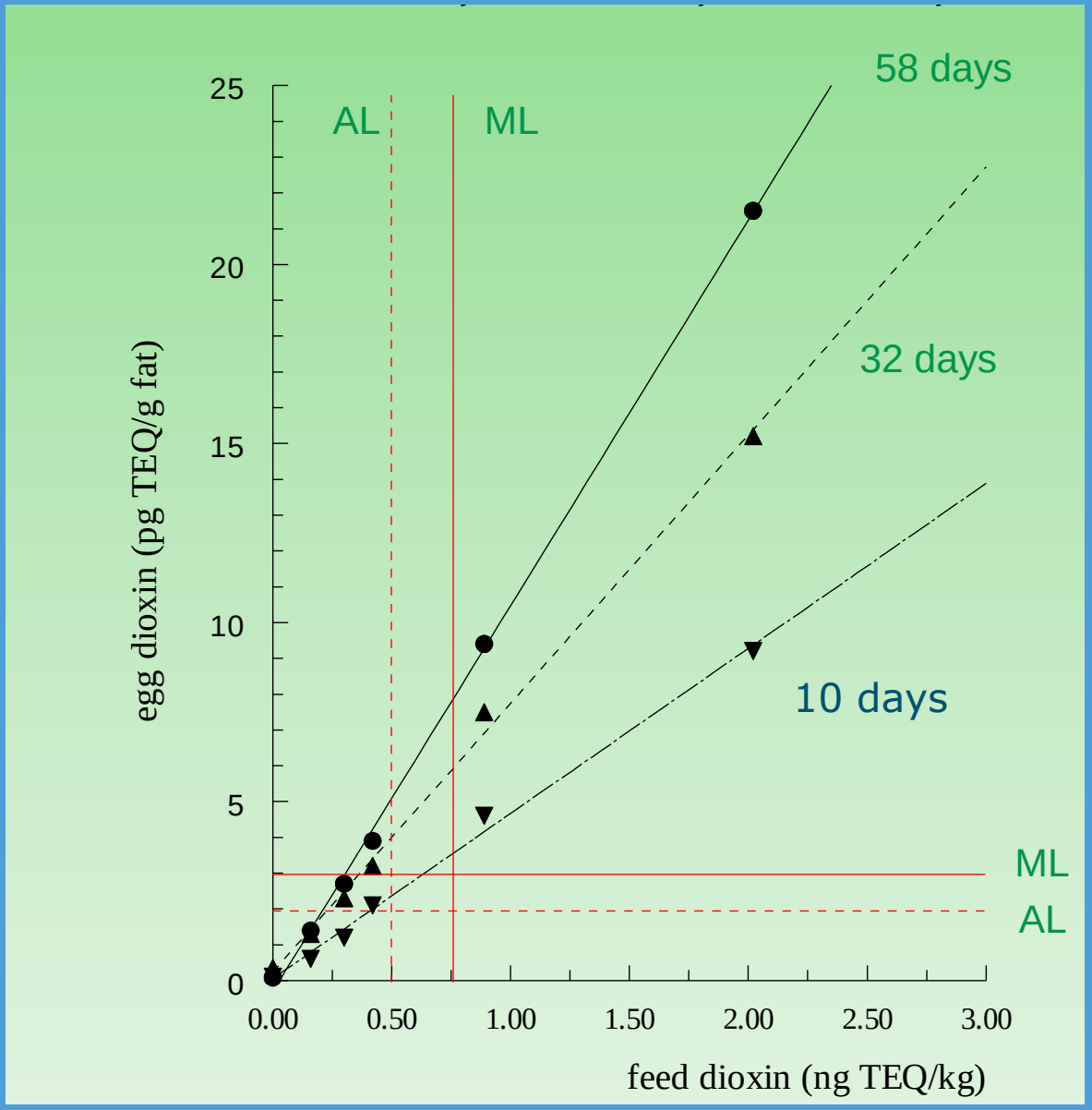
Feed egg limits relationship



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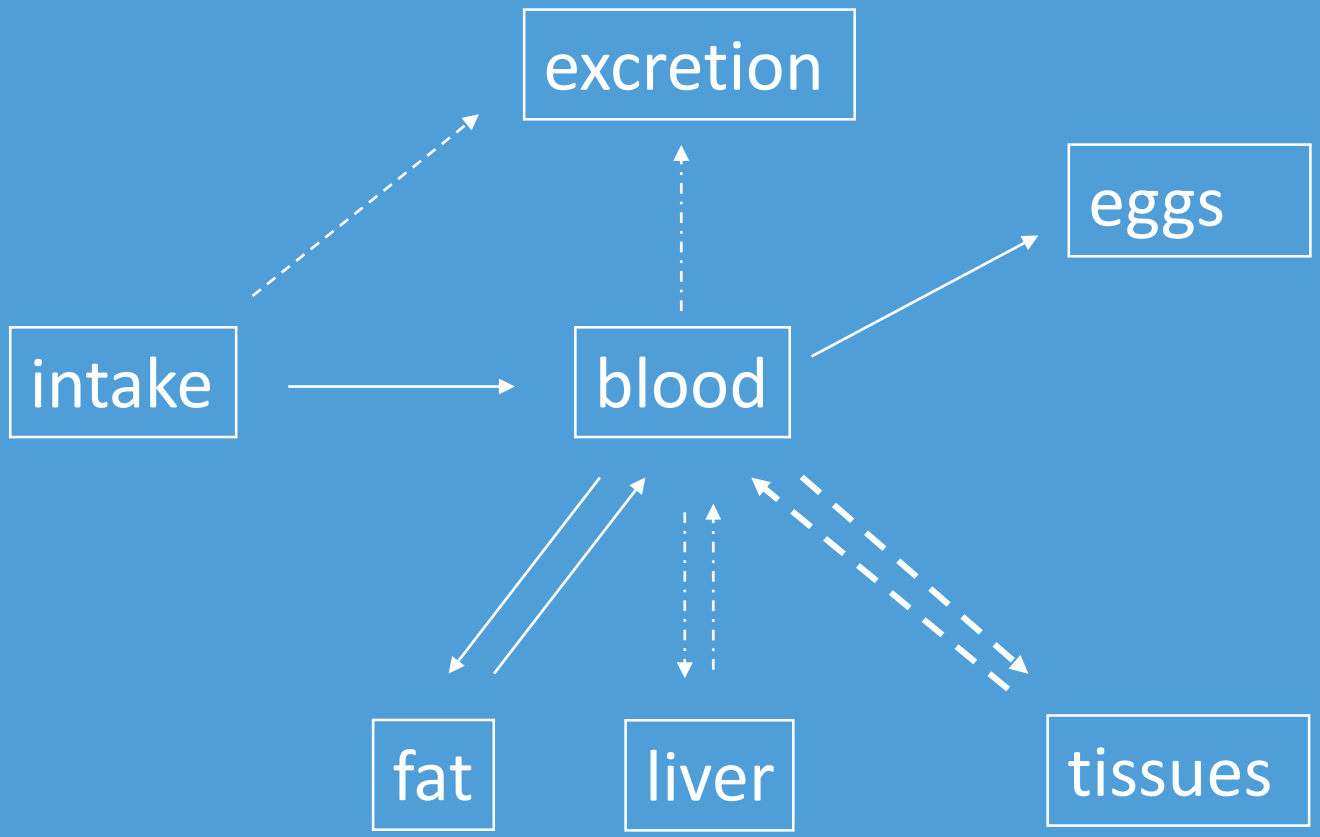
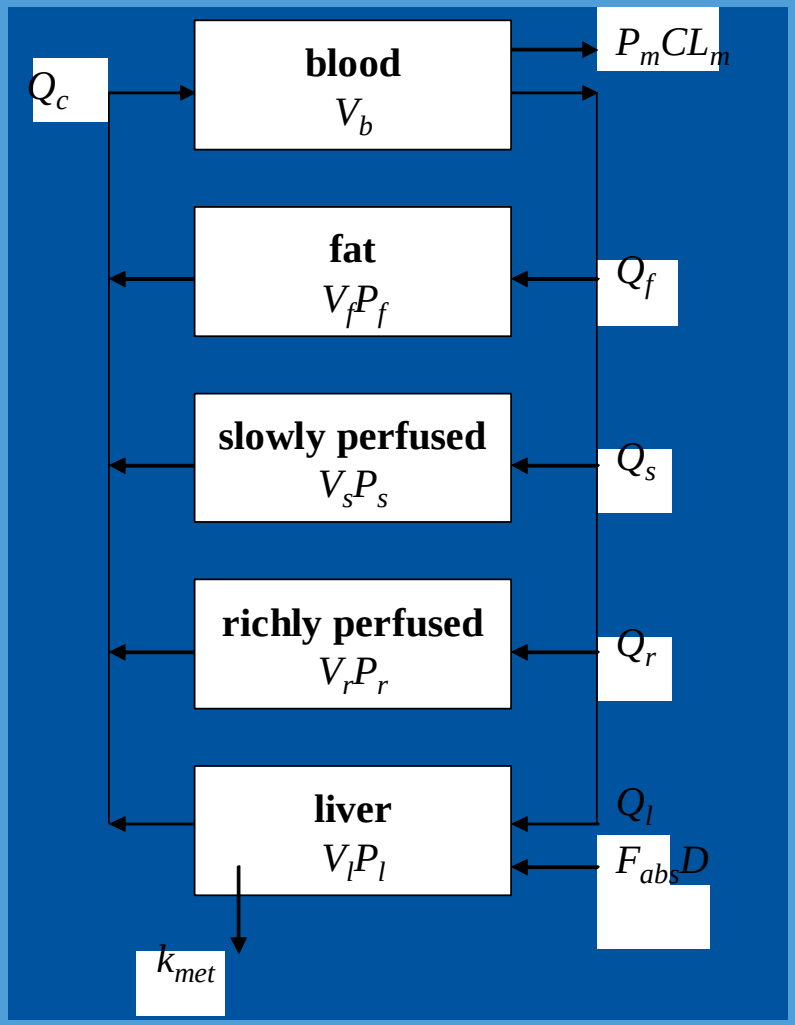
PBPK-modelling laying hen

- Based on physiology in the animal
  - Absorption, excretion
  - Exchange between blood and tissues
  - Excretion in eggs
  - Only relevant tissues modelled
- Normally modelled on TEQ (as if only one compound)

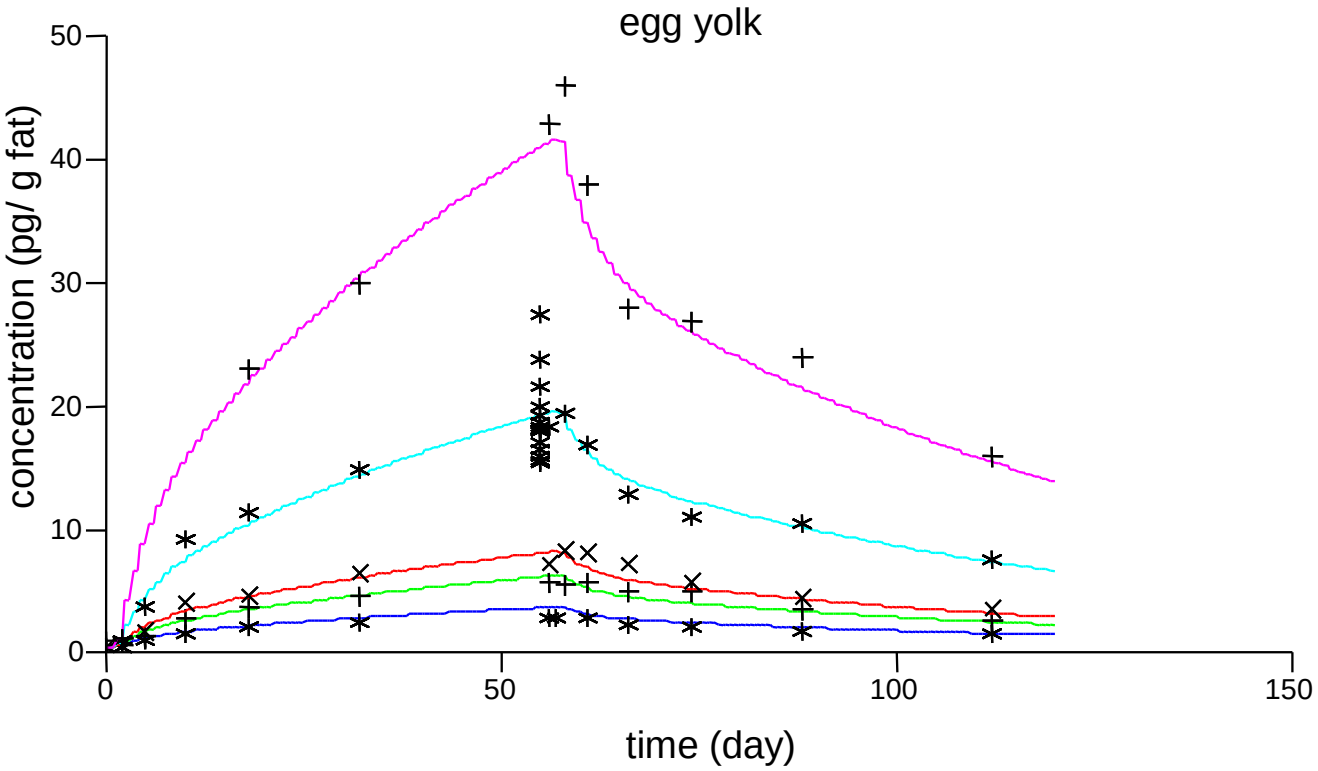
Kinetics in laying hens



PB-PK Models

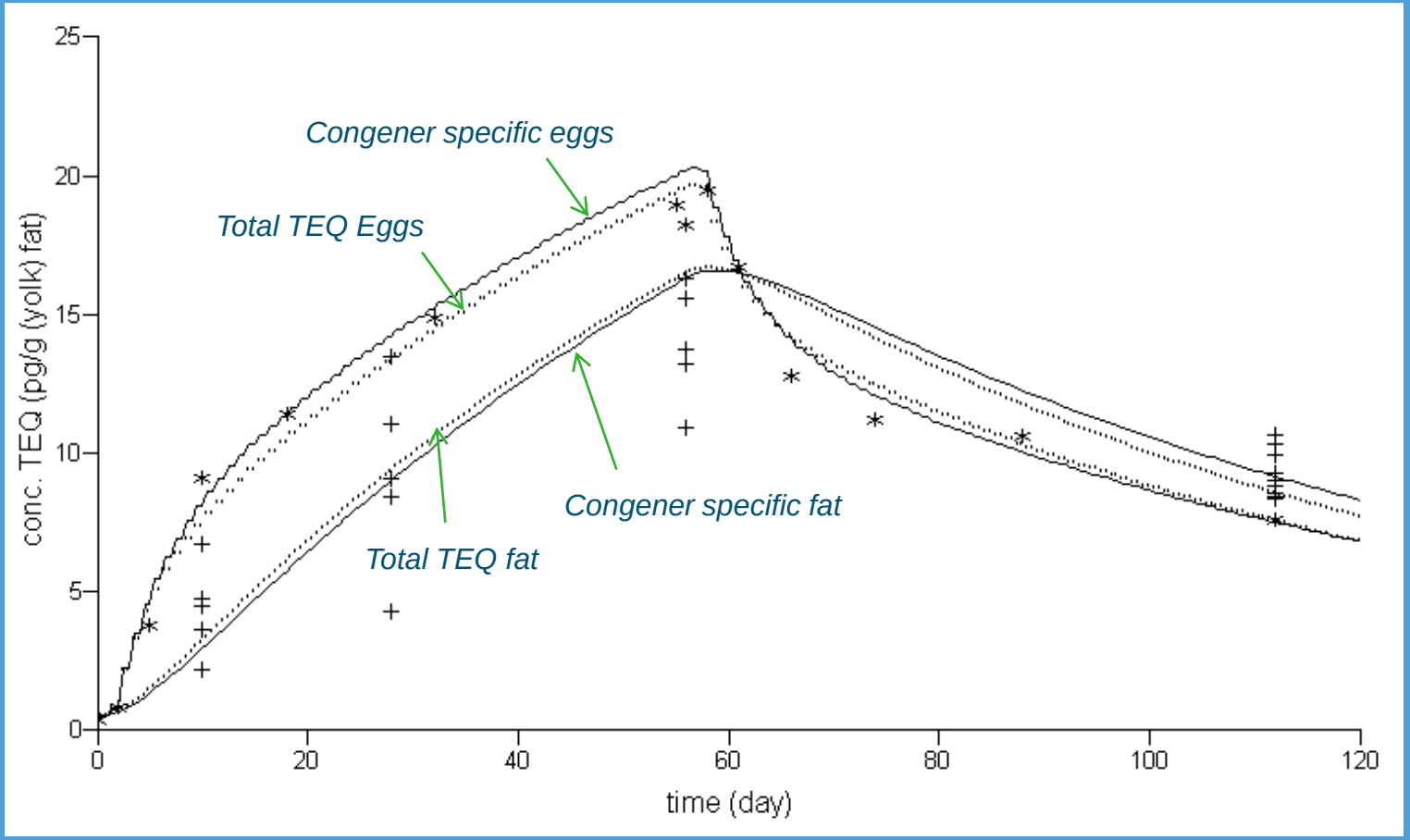

blood
V_b
fat
$V_f P_f$
slowly perfused
$V_s P_s$
richly perfused
$V_r P_r$
liver
$V_l P_l$
Q_c
$P_m CL_m$
Q_f
Q_s
Q_r
Q_l
$F_{abs} D$
k_{met}

TEQ-based modelling (sum dioxins, dl-PCBs)



Congener specific modelling

- Differences in absorption, metabolism and carry-over between congeners
  - Also depends on animal species
- Therefore also modelling per congener
  - Laying hen
  - Dairy cow
- Integration RIKILT/RIVM models in CORAM
  - Will be on website



Lower chlorinated congeners drive TEQ: no big differences

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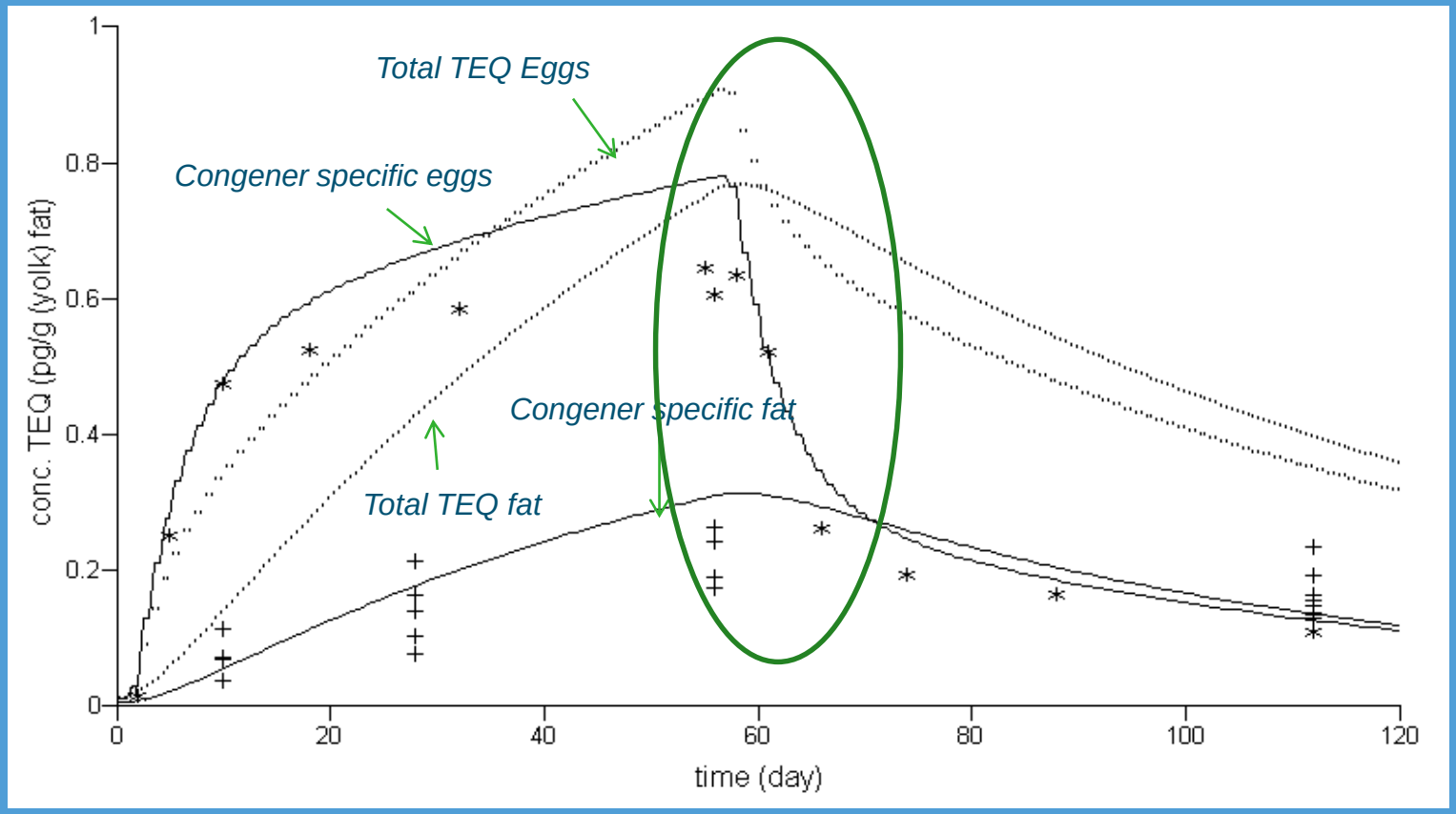

Higher chlorinated congeners drive TEQ: big differences

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Sheep

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Problem

- Often very high levels of dioxins in livers of sheep
- EFSA opinion: potential risk for the consumer
- Very little information on relation between intake and levels in livers and fat
- Therefore carry-over study with EU-QSAFFE project

Levels of dioxins and dl-PCBs in livers and meat of sheep (EFSA 2010)

	n	Min	P5	P50	P90	P95	P99
Dioxins							
Liver	332	0.3	1.0	7.8	36.1	55.5	92.6
Meat/fat	175	0.1	0.2	0.5	1.5	2.3	3.9

Based on TEFs 1998; ML liver until 2012 was 6 and 12 pg TEQ/g fat, for meat and fat: 3.0 and 4.5 pg TEQ/g fat

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Meat/fat	175	0.1	0.2	0.5	1.5	2.3	3.9
dl-PCBs							
Liver	332	0.1	0.2	5.8	21.9	41.6	110.4
Meat/fat	175	0.1	0.2	0.9	2.5	3.3	9.7
Sum TEQ							
Liver	332	0.5	1.4	14.3	61.1	98.1	167.5
Meat/fat	175	0.2	0.3	1.4	3.5	5.5	10.6

Based on TEFs 1998; ML liver until 2012 was 6 and 12 pg TEQ/g fat, for meat and fat: 3.0 and 4.5 pg TEQ/g fat

QSAFFE Task 4.1 Carry-over study with sheep

- Study with lambs performed by BfR, Berlin
- Sheep fed with slightly contaminated feed followed by clean feed (feed delivered by RIKILT)
- Analysis of samples by RIKILT
- Modelling of data by RIVM (congener specific)

Carry-over study with sheep

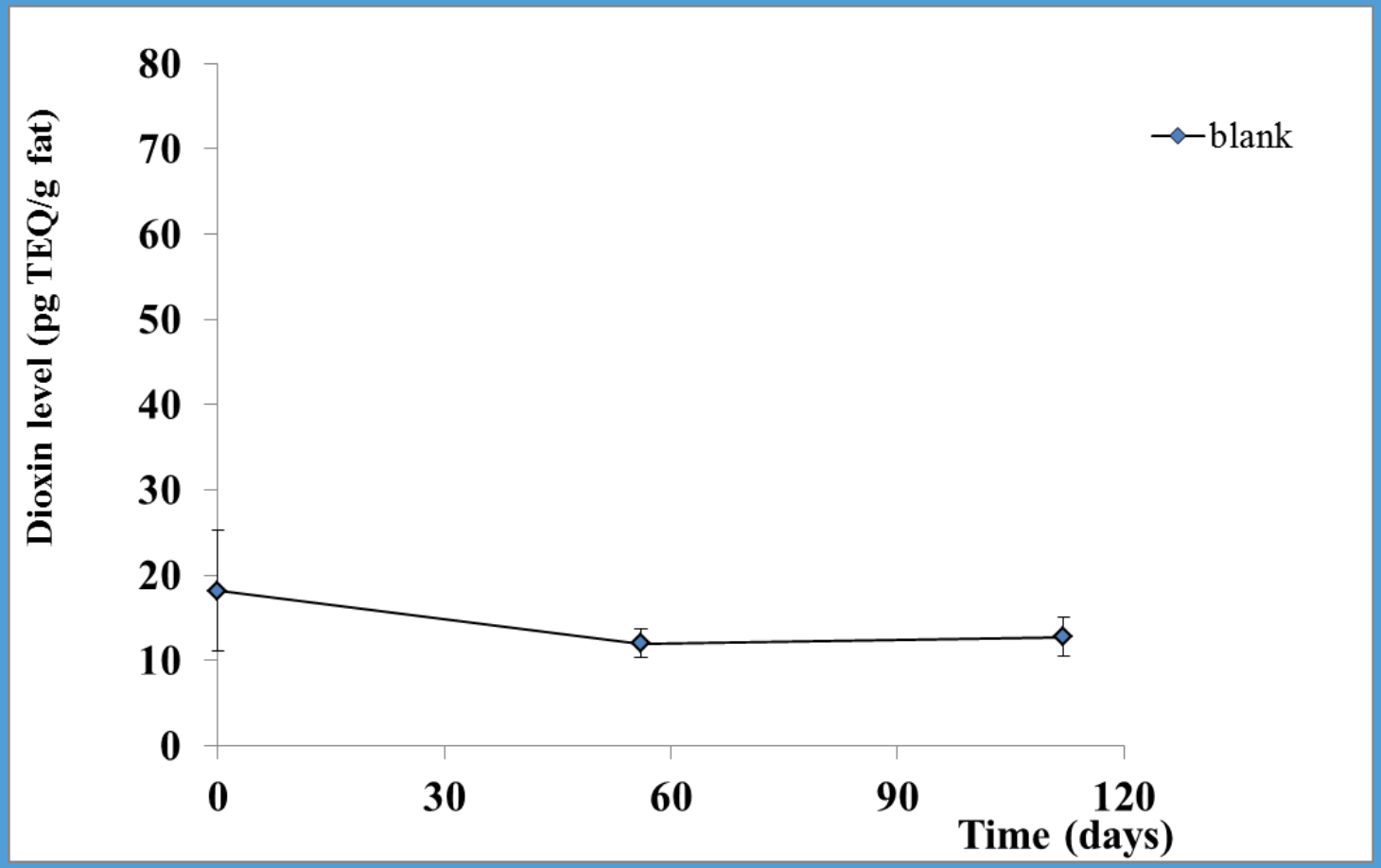
- Study with blackhead lambs performed by BfR Berlin
- Sheep fed with contaminated grass followed by clean grass (both from NL; contaminated grass from flood plain)

Grass

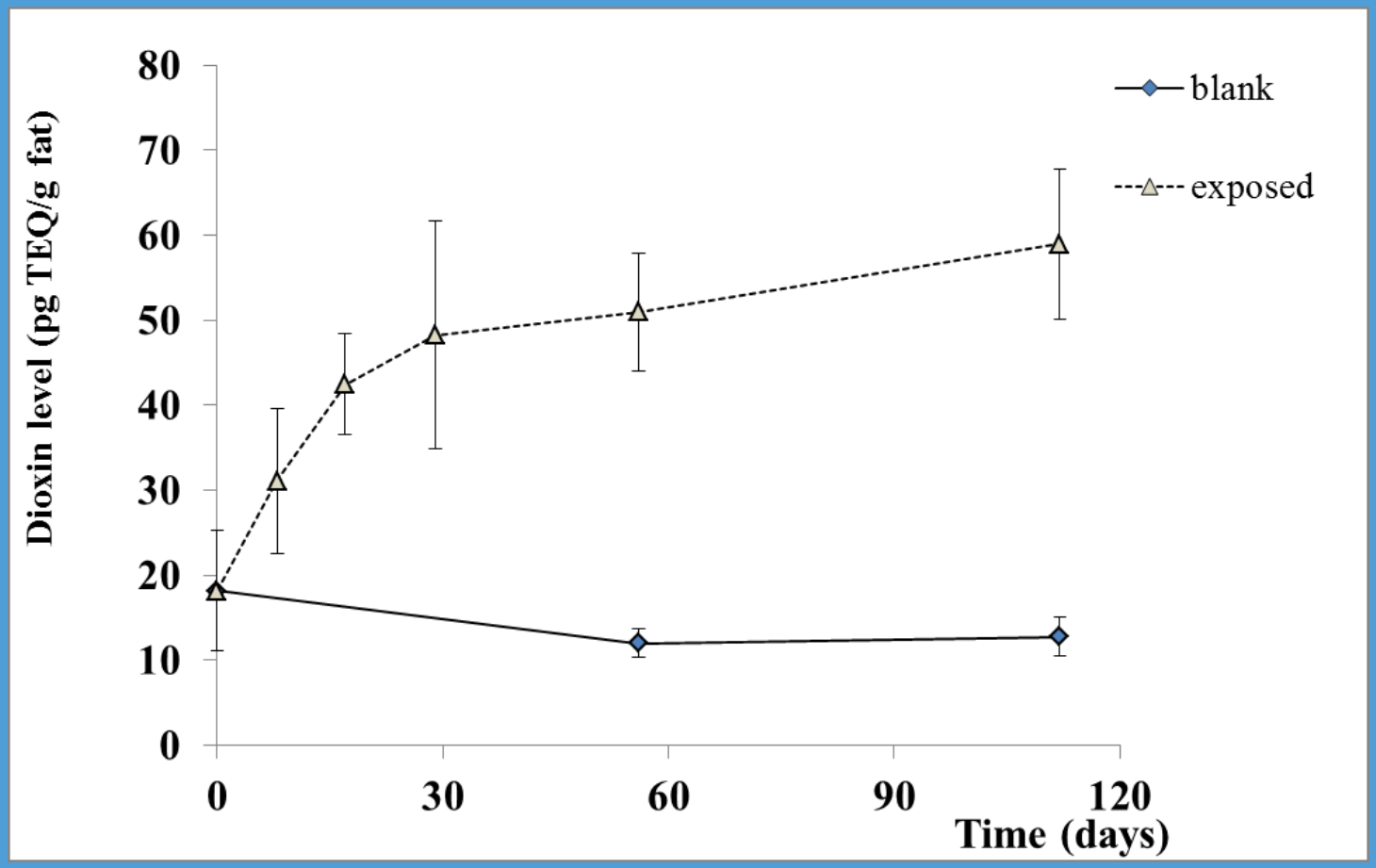
	Dioxins pg TEQ/g dm	dl-PCBs pg TEQ/g dm	Sum pg TEQ/g dm	ndl-PCBs µg/kg dm
Clean	0.27 (0.26)	0.06 (0.06)	0.33 (0.32)	0.45 (0.45)
Contaminated	1.71 (1.71)	0.32 (0.32)	2.04 (2.02)	2.33 (2.33)

- Contaminated grass derived from river bank of IJssel river. Contamination due to high levels in soil partly ending up in the grass during harvesting
- ML: 0.75/1.25 ng TEQ/g dm, 10 µg/kg dm for ndl-PCBs
- Focus on dioxins

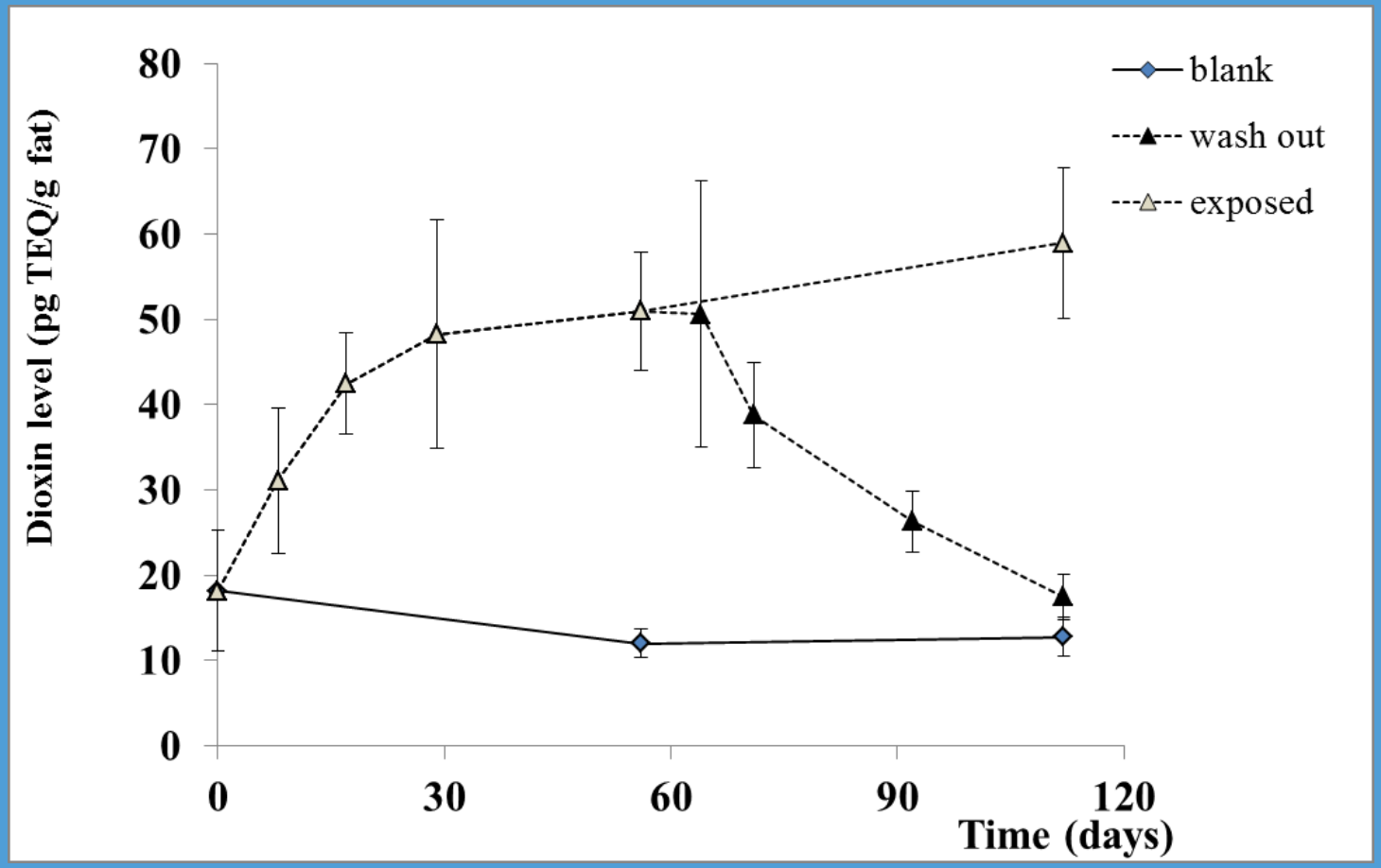
Dioxins in liver (blank)



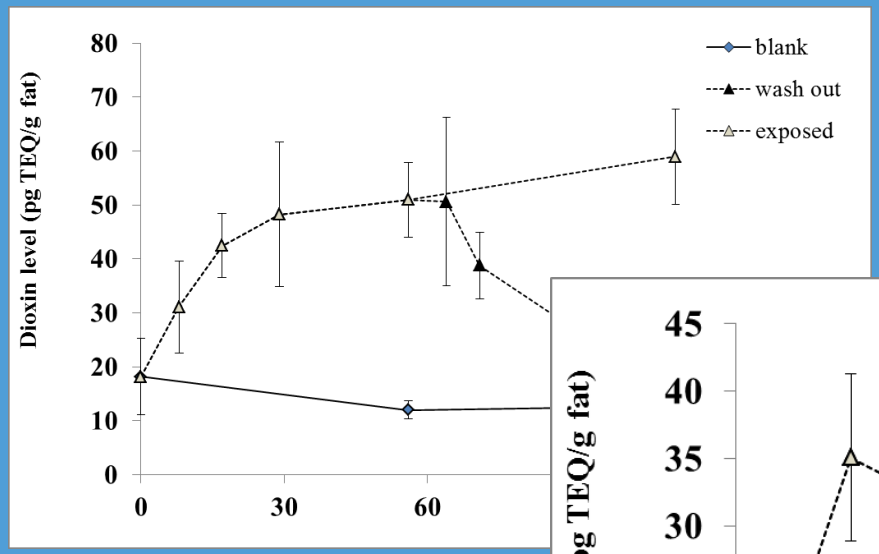
Dioxins in liver (exposed for 112 d)

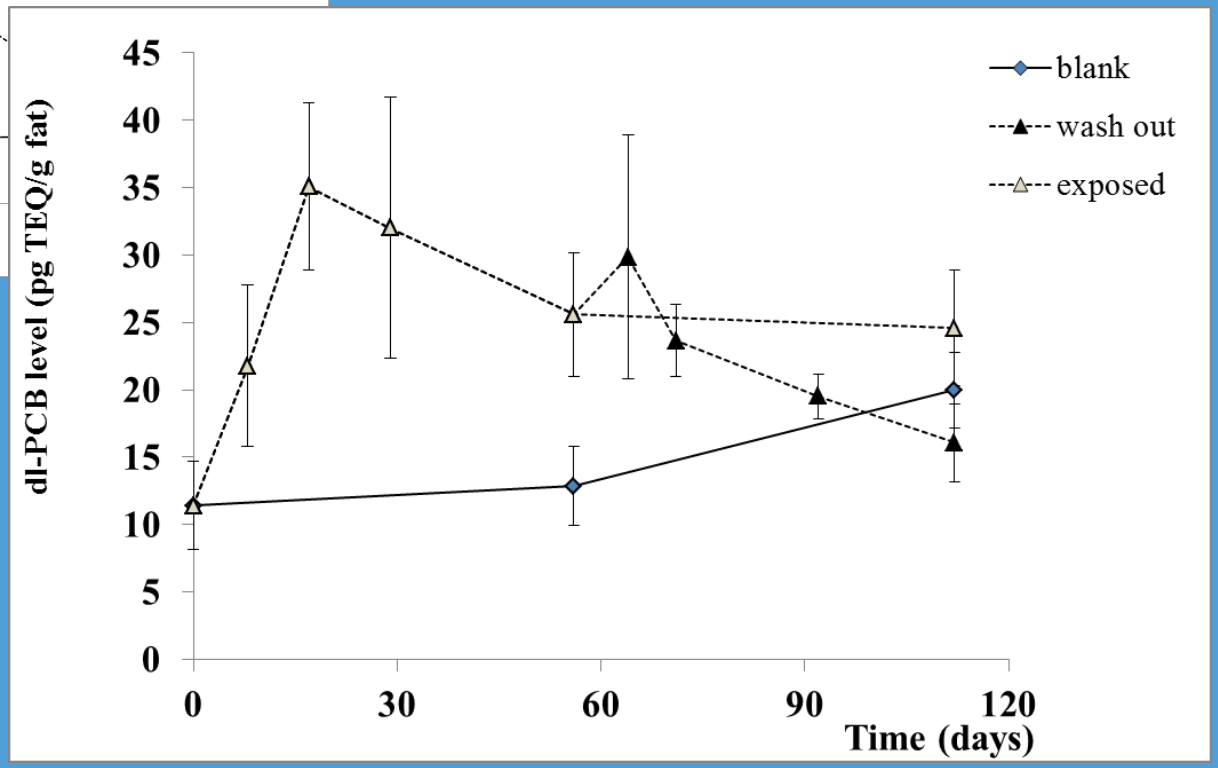


Dioxins in liver (wash-out after 56 days)



Dioxins and dl-PCBs in livers





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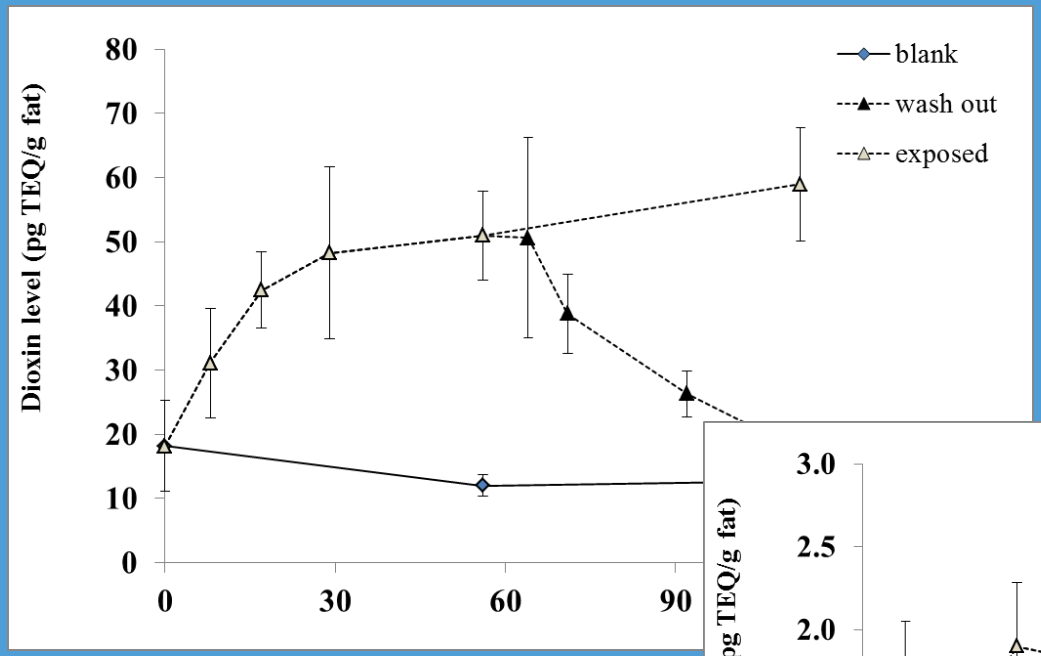
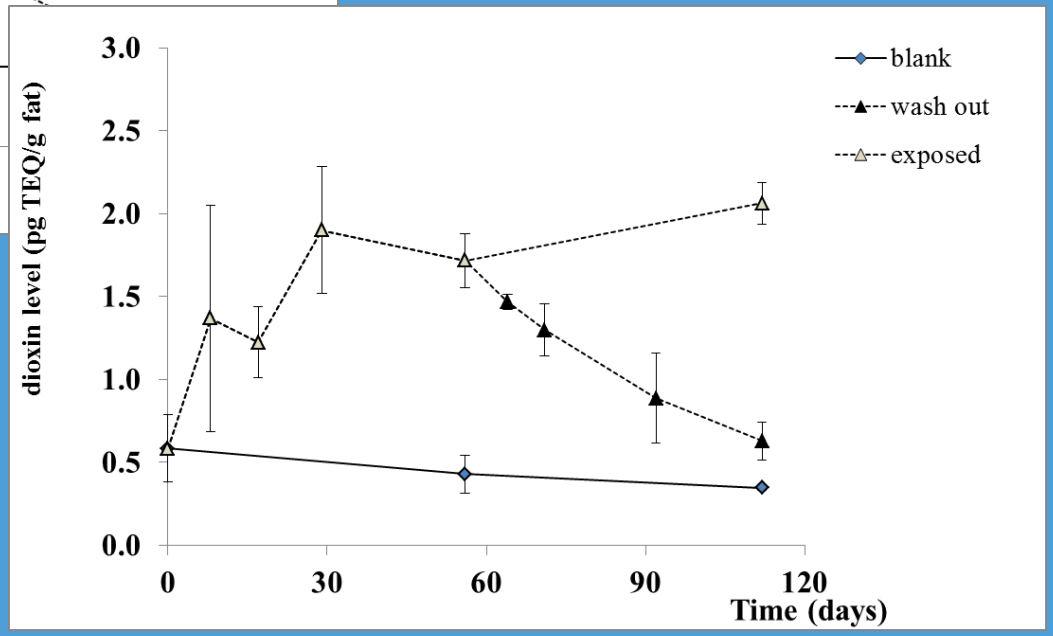
Dioxins in livers versus kidney fat



Levels in liver on lipid base 30 times higher



MLs liver 4.5, fat 2.5 pg TEQ/g fat

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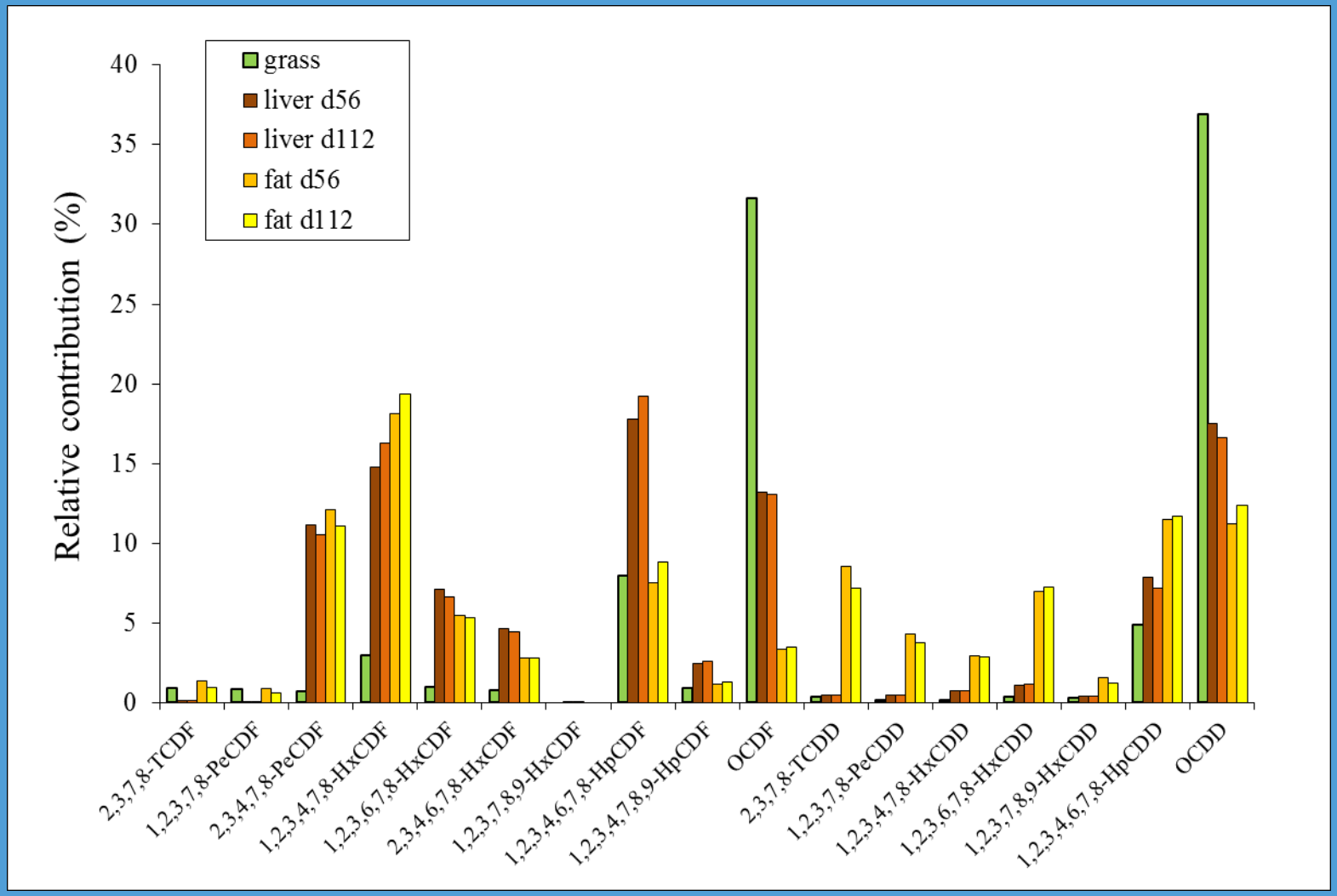
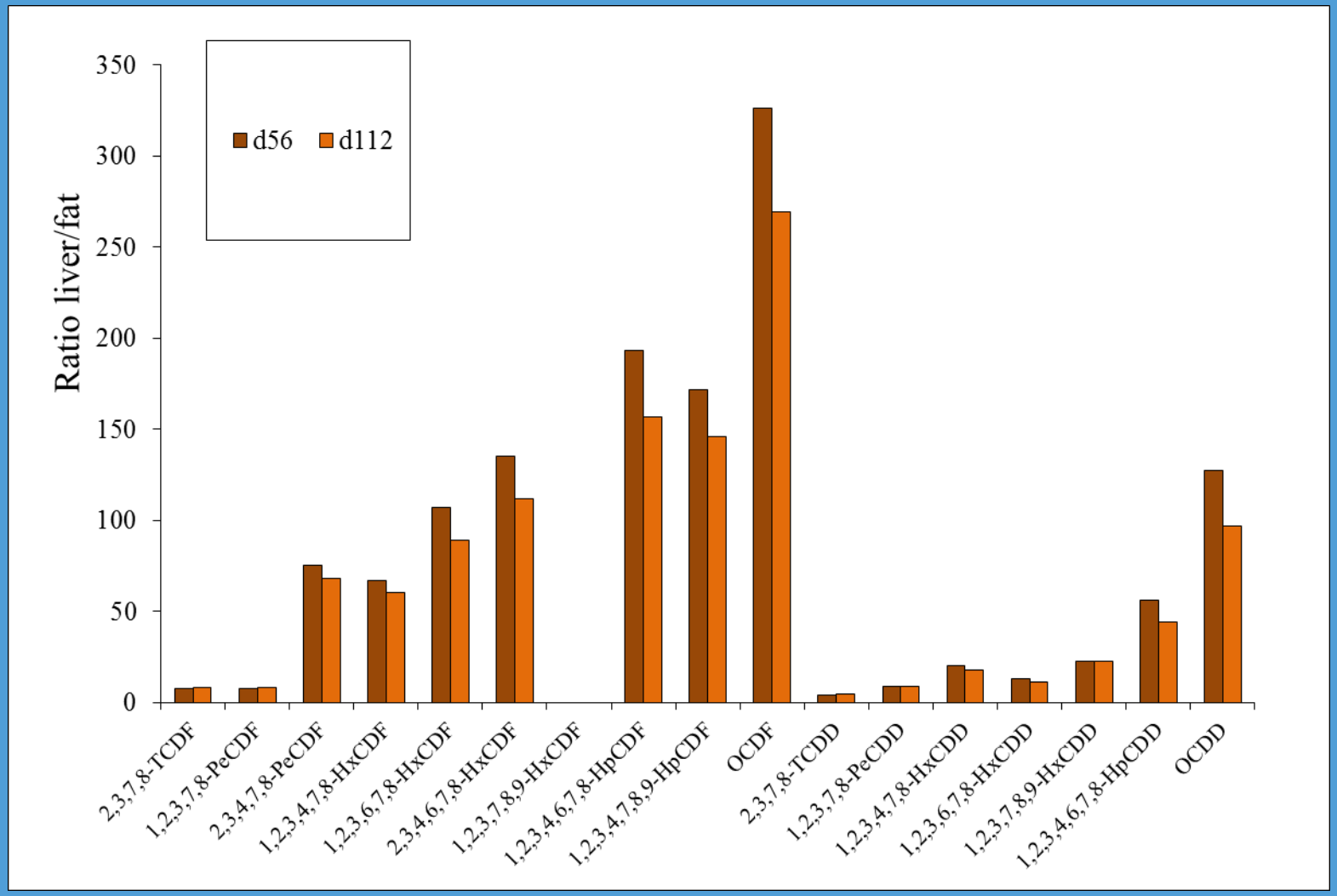
Dioxin patterns in grass, liver and kidney fat



Congener ratios liver/kidney fat



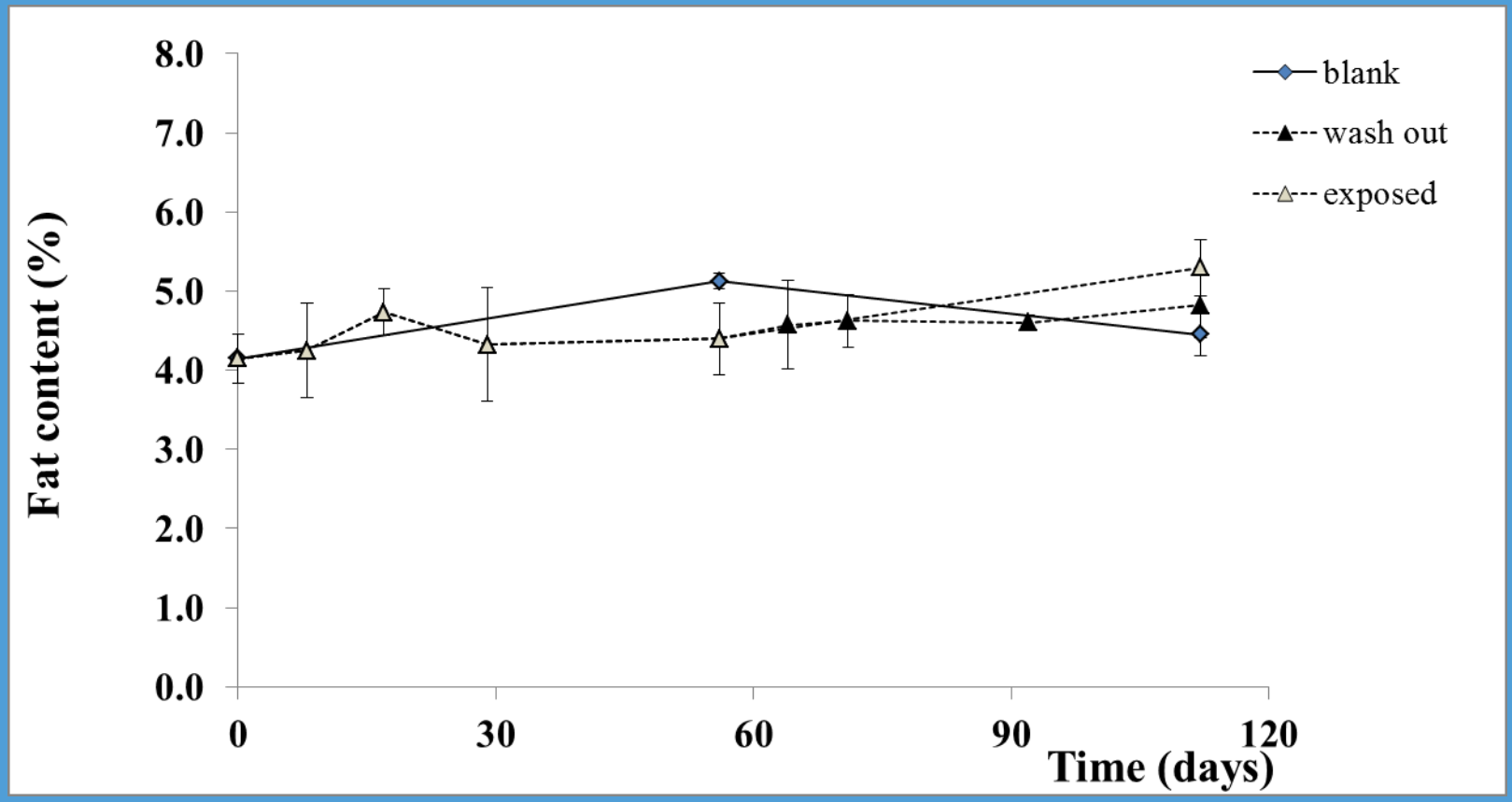
What about meat?

- In general distribution over fat compartments is thought to be equal
  - So levels in kidney fat should be similar as in meat fat
- Based on EFSA database this appears not to be correct
  - But data from different countries, so not the same animals
- What about the sheep in our study?
- Ratio meat fat/kidney fat:
  - 0.9 ± 0.1 at day 56, 0.8 ± 0.1 at day 112

Expression livers on fresh weight base

- Highest levels dioxins and total TEQ around 59 and 84 pg TEQ/g fat
    - Current EU MLs: 4.5 and 10 pg TEQ/g fat

Fat content



- Levels very stable around 4-5%

Expression livers on fresh weight base

- Highest levels dioxins and total TEQ around 59 and 84 pg TEQ/g fat
  - Current EU MLs: 4.5 and 10 pg TEQ/g fat
- Based on fresh weight around 3 and 4 pg TEQ/g liver
  - New proposed MLs: 1.25 and 2 pg TEQ/g weight

Pigs

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Pigs

- Rapid increase in weight, 0-110 kg in 6 months
  - Also increase in fat content
- Some PCDFs seem metabolized
- One study by RIKILT/ASG/RIVM
  - Using feed from Belgium crisis
  - Paper shows BCFs (level in fat/level in feed)
- Modelled at a later stage related to gelatine incident

Levels in pigs fed Belgium feed (15x diluted) for 7 d at age 3 mo, followed by clean feed up to 12 wks

Week	dioxins	non-ortho PCBs	mono-ortho PCBs	total	indicator PCBs	Ratio indicator PCBs/dioxins
		(pg TEQ/g fat)			(µg/g fat)	(x1000)
0	26.1	15.3	81.9	123.3	3.48 ± 0.49	133
1	21.8	10.3	63.0	95.1	2.65 ± 0.33	122
2	15.0	6.4	48.1	69.5	2.01 ± 0.26	134
4	7.4	3.0	29.4	39.8	1.25 ± 0.17	168
8	3.3	1.3	17.7	22.3	0.76 ± 0.12	230
12	1.3	0.6	10.3	11.9	0.45 ± 0.06	357

$TEFs_{1998}$

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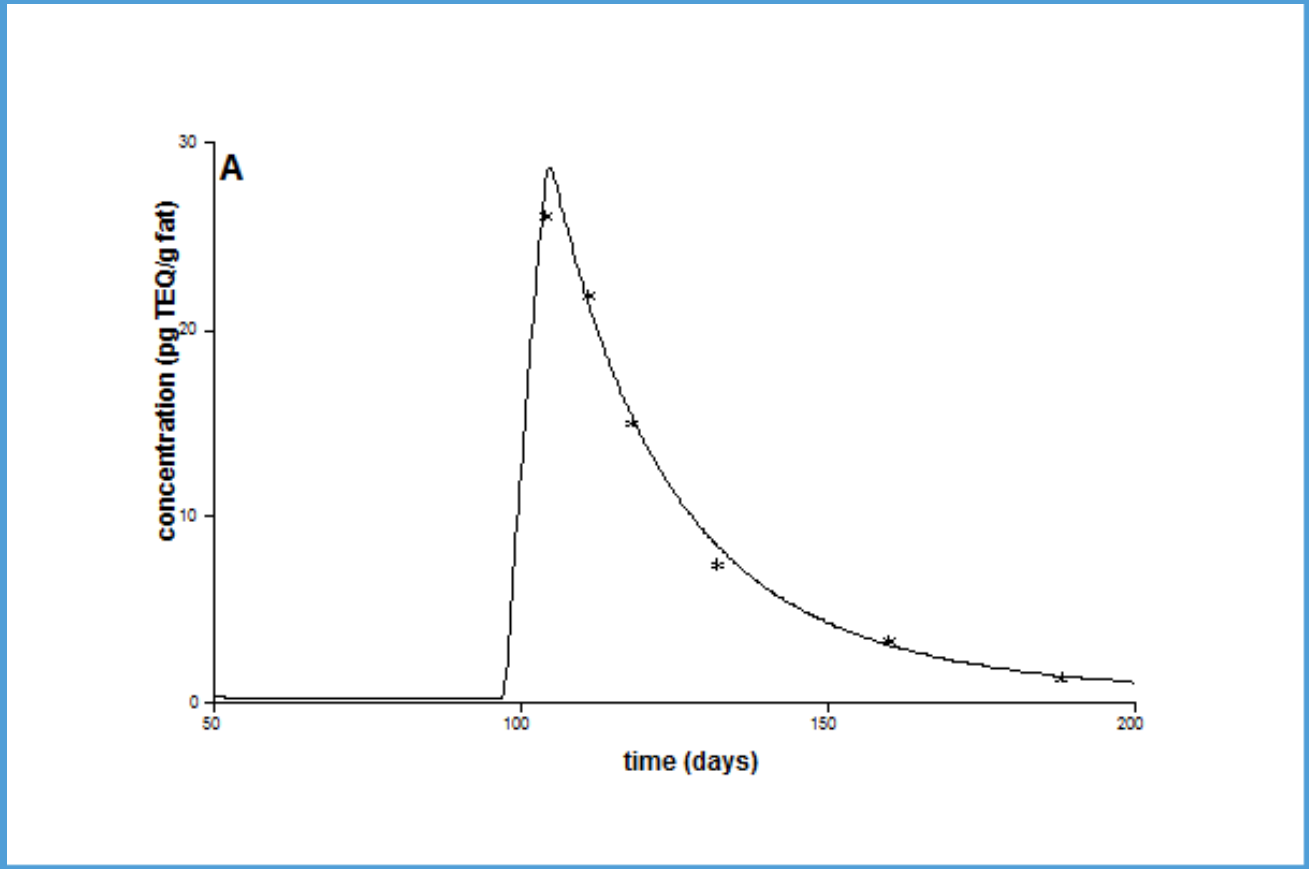
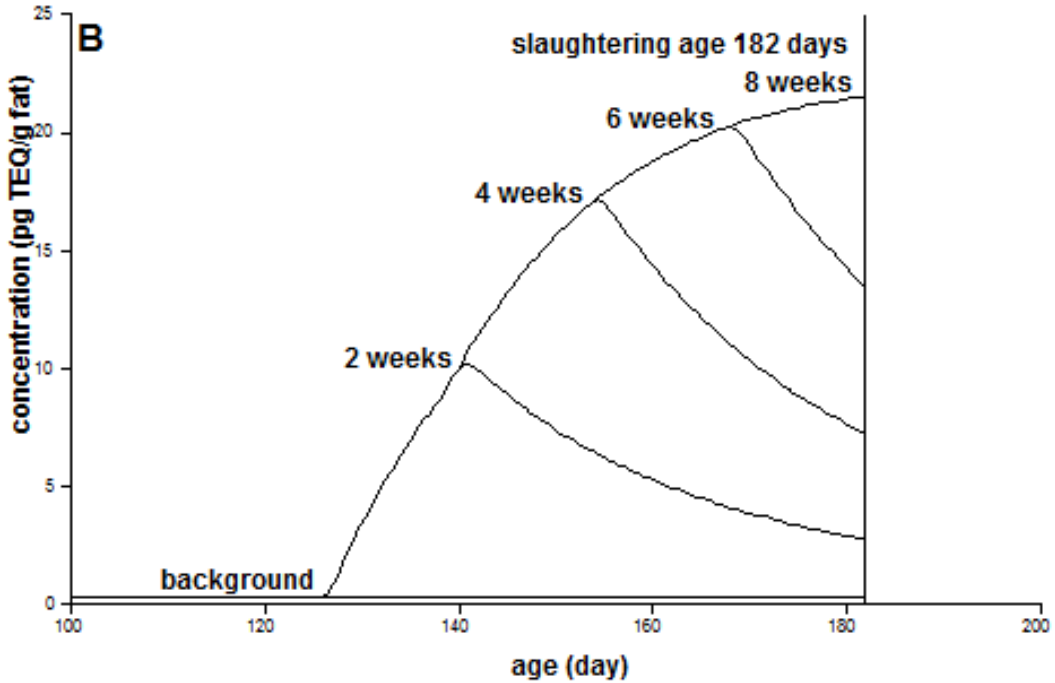
PBPK model



Modelled data from pig study, taking into account the growth of pigs

Predictions based on model (gelatine incident, 2006)



Levels based on 2, 4, 6 or 8 wks contaminated feed (8.4 ng TEQ/kg), starting at age 126 d; followed by clean feed

Broilers

- Also broilers included in study
- Like pigs rapid growth, 0-2 kg in 6 weeks
- Treatment at age of 3 wks for 1 week
- Withdrawal periods of 0, 1 or 3 wks
- BCFs determined
    - Ratio in fat compared to feed
    - (see Hoogenboom et al. 2004)
- Data not modelled in PBPK

Study on broilers with Belgium feed

(15x diluted)

Week	dioxins	non-ortho PCBs	mono-ortho PCBs	total	Indicator PCBs	ratio indicator PCBs/diox
		(pg TEQ/g fat)			(µg/g fat)	(x1000)
Feed	48	20	96	164	3.8	79
0	102	84	216	402	6.2	61
1	55	41	121	217	3.2	58
3	26	22	61	109	1.5	58

feed in ng TEQ/kg dm; $TEFs_{1998}$

High levels of dioxins and PCBs in meat and livers of pigs, goats, sheep and cows from Curaçao

Ron Hoogenboom, Guillaume ten Dam, Stefan van Leeuwen, Harry van Egmond, Jennyfer Nicolina, Arnold Dwarkasing

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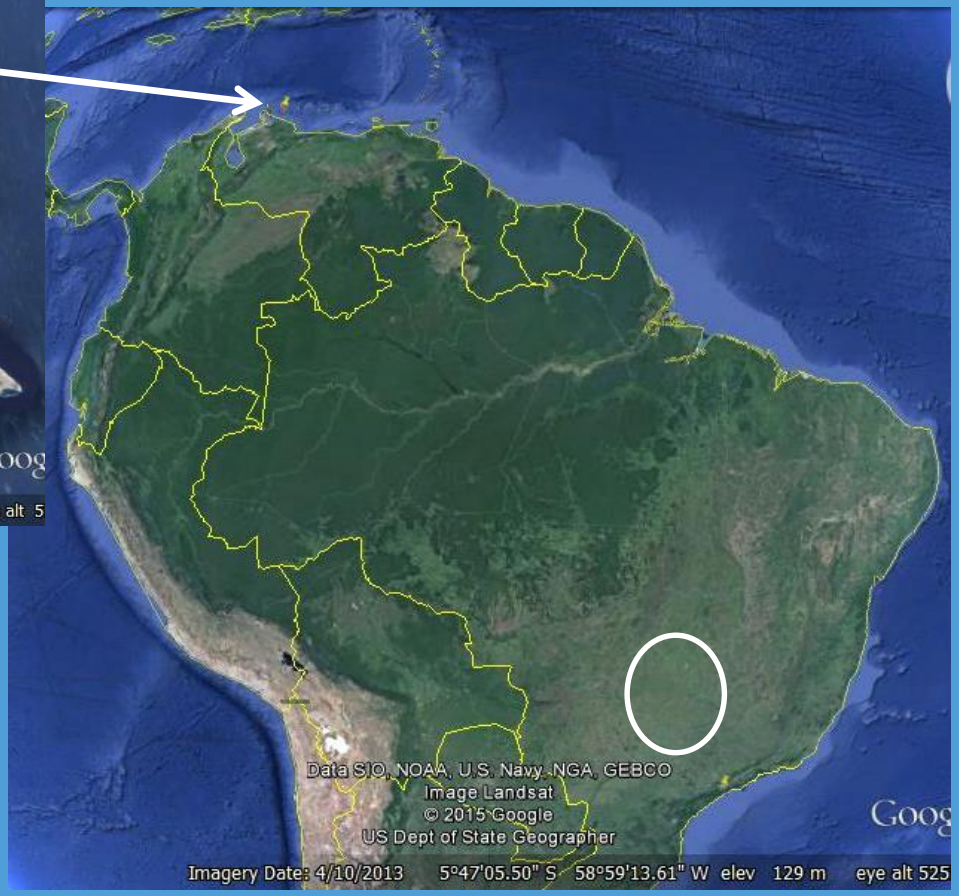
Curaçao



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Samples from Curaçao

- End of 2014, request to analyze pork fat samples
- Regarding expected low levels, CALUX offered, with confirmation by GC/HRMS, if required (not expected)
- 17 samples in first shipment

First results pig fat

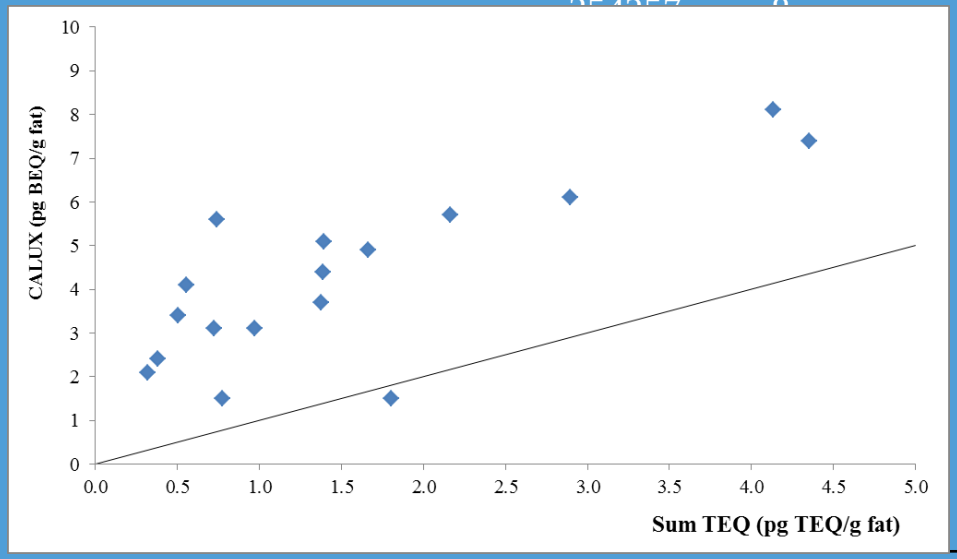
- All but 1 suspected
- Confirmation required

RIKILT nr	Nr	CALUX pg BEQ/g	PCDD/Fs pg TEQ/g	dl-PCBs pg TEQ/g	Sum pg TEQ/g	ndl-PCBs ng/g
354250	1	**4.1**				
354251	2	**3.7**				
354252	3	**<0.5**				
354253	4	**1.5**				
354254	5	**2.4**				
354255	6	**4.4**				
354256	7	**3.4**				
354257	8	**1.5**				
354258	9	**5.7**				
354259	10	**8.1**				
354260	11	**5.6**				
364940	11	**5.1**				
354261	12	**3.1**				
354262	13	**3.1**				
354263	14	**7.4**				
354264	15	**6.1**				
354265	16	**4.9**				
354266	17	**2.1**				

First results pig fat

- Levels relatively high
- Number of non-compliants
- Overestimation by CALUX

RIKILT nr	Nr	CALUX pg BEQ/g	PCDD/Fs pg TEQ/g	dl-PCBs pg TEQ/g	Sum pg TEQ/g	ndl-PCBs ng/g
354250	1	**4.1**	0.38	0.18	0.56	10.2
354251	2	**3.7**	1.04	0.34	1.38	4.04
354252	3		0.24	0.16	0.40	1.21
354253	4	**1.5**	0.42	1.39	**1.80**	**87.6**
354254	5	**2.4**	0.29	0.09	0.38	4.82
354255	6	**4.4**	1.12	0.17	1.39	6.18
354256	7	**3.4**	0.35	0.16	0.51	2.26
		1.5	0.59	0.18	0.77	8.27
		5.7	**1.78**	0.38	**2.16**	5.48
		8.1	**2.91**	1.22	**4.13**	20.2
		5.6	0.56	0.18	0.74	5.45
		5.1	1.17	0.22	1.39	9.32
		3.1	0.55	0.18	0.73	5.67
		3.1	0.70	0.27	0.97	4.95
		7.4	**2.95**	1.40	**4.35**	17.2
		6.1	**1.85**	1.05	**2.90**	11.0
		4.9	1.12	0.55	**1.67**	9.69
		2.1	0.22	0.10	0.32	1.97



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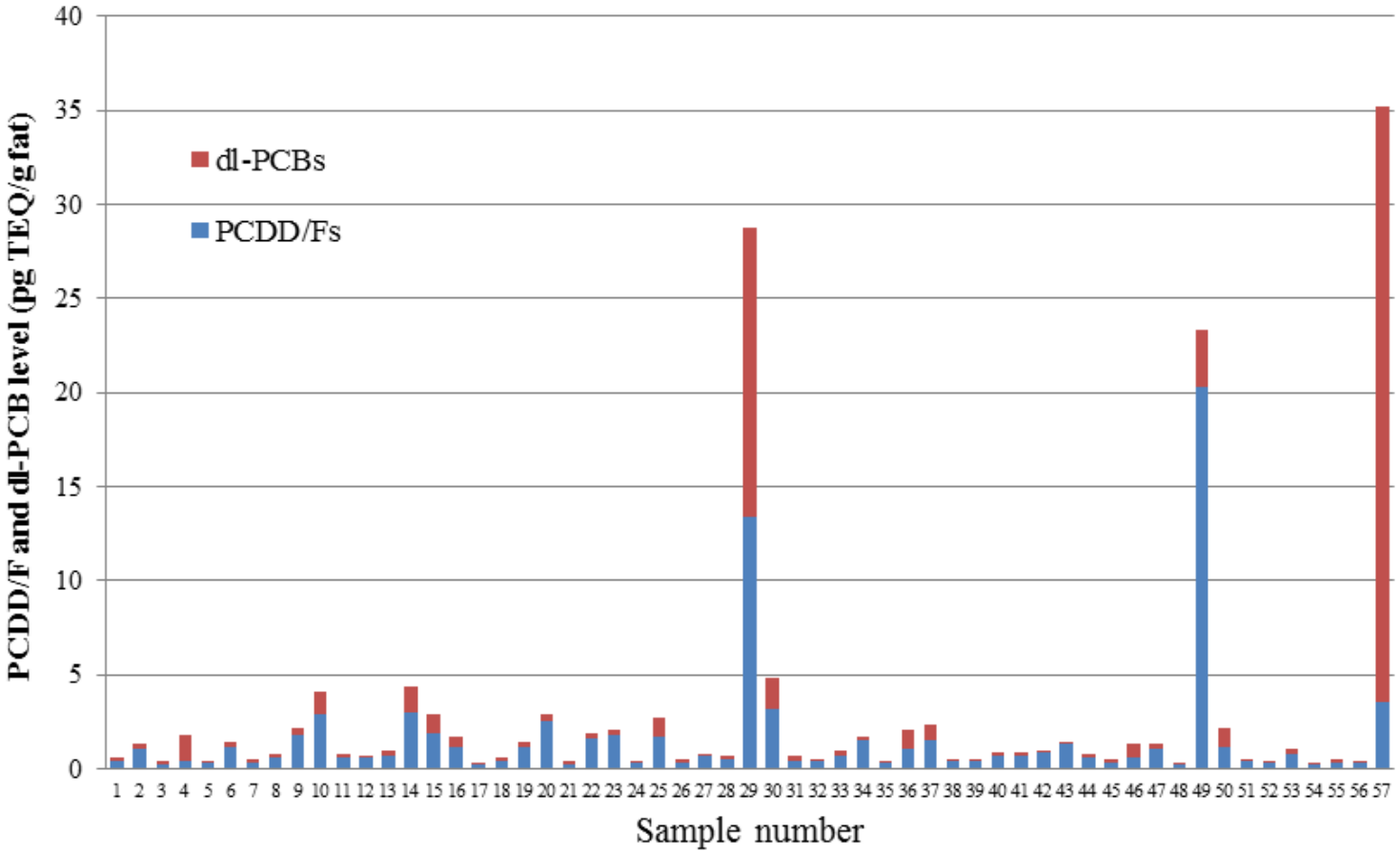
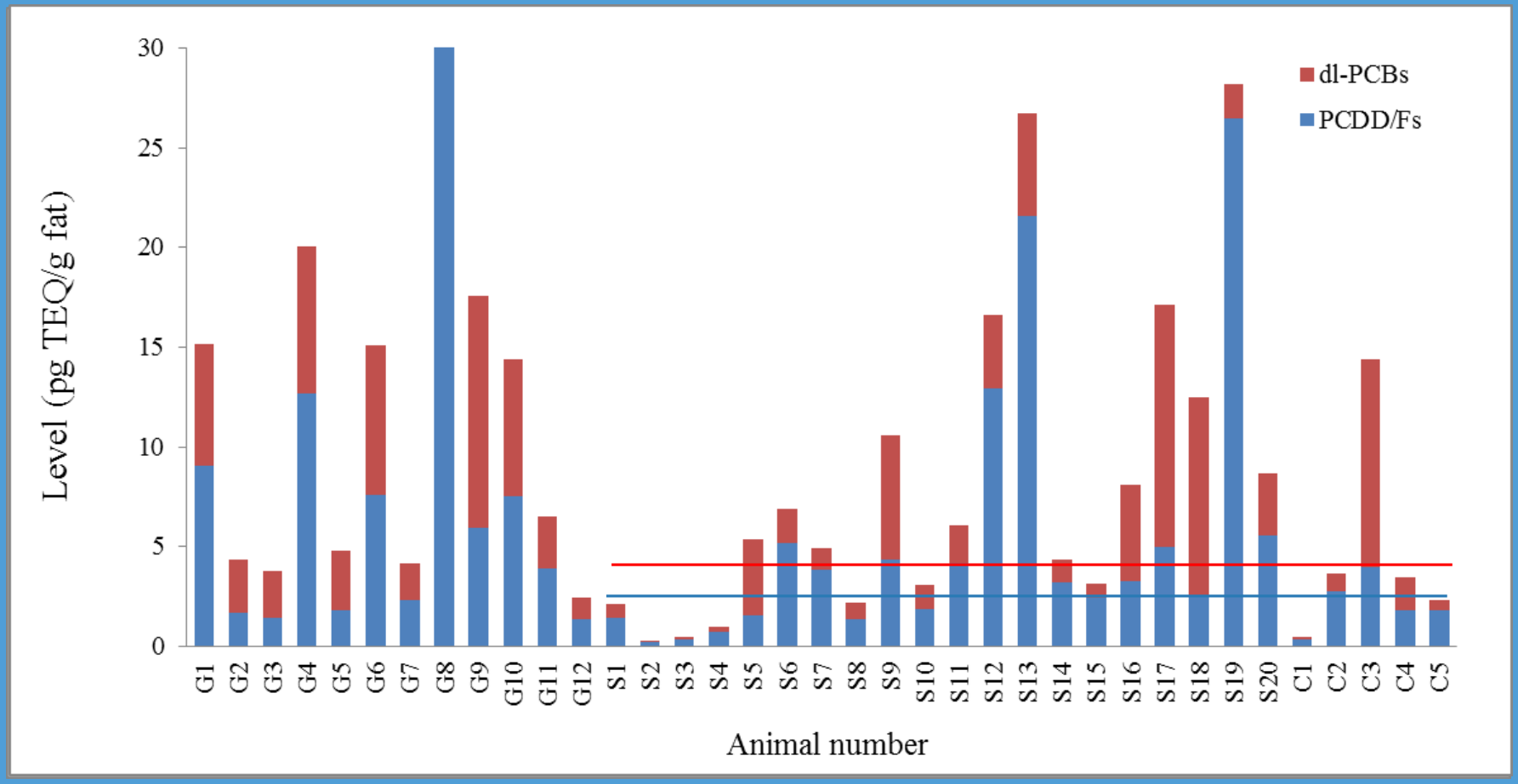
More pig fat samples analyzed (GC/MS)



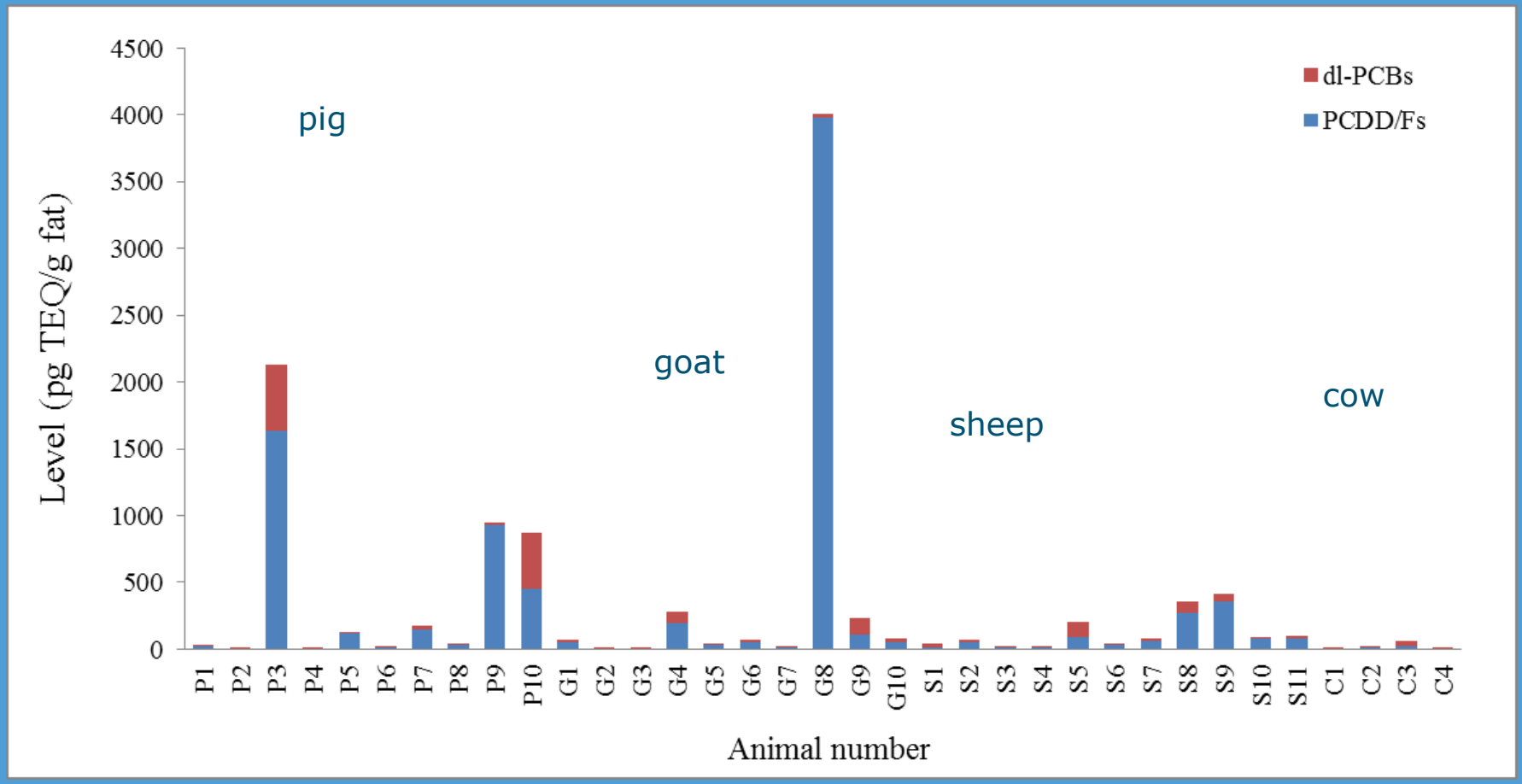
Also goats, cows, sheep



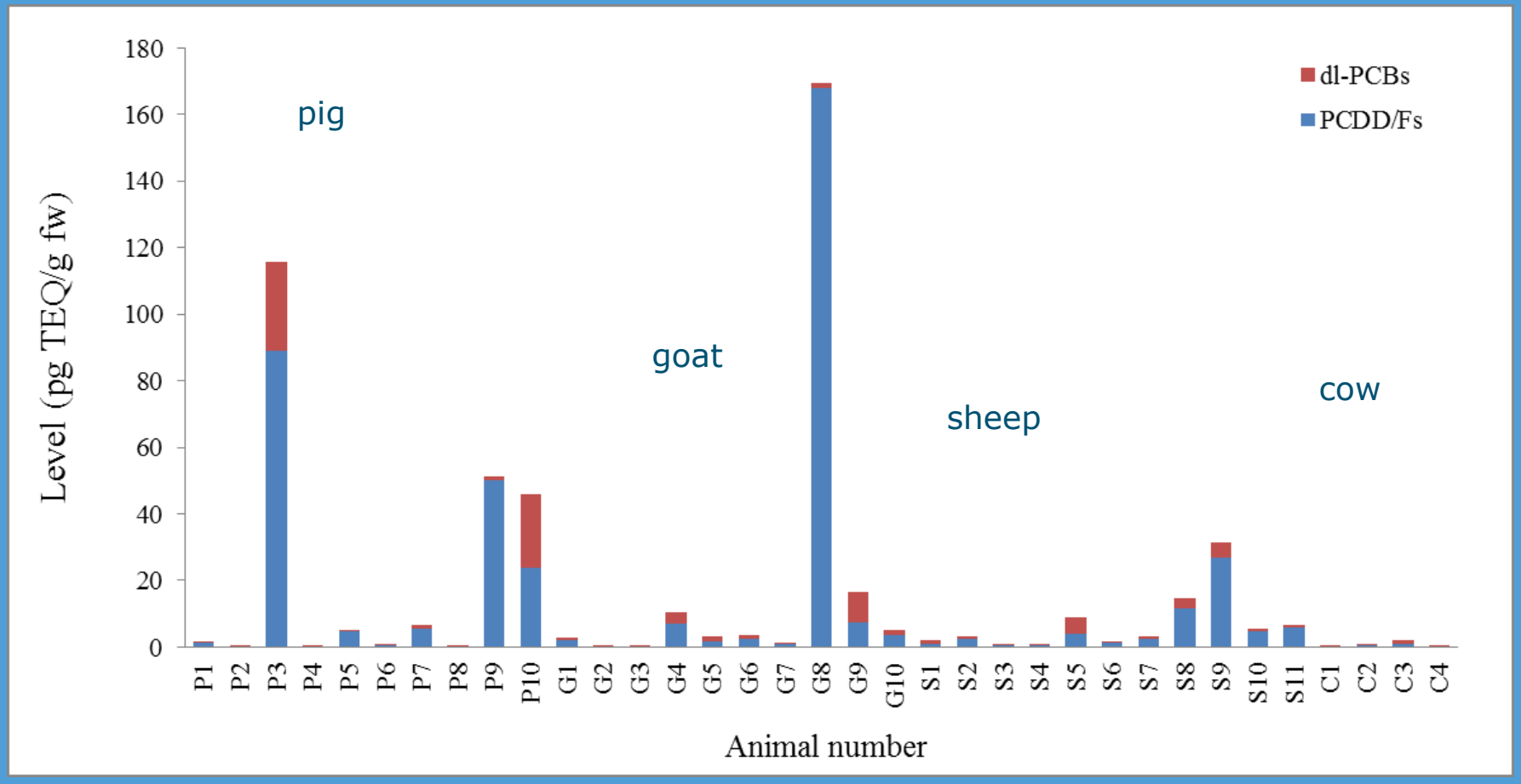
What about livers (sequestration)?

- Dioxins and dl-PCBs bind to liver proteins (CYP 1A2)
  - Lipid-based levels higher in liver than fat
  - Congener-specific
- Especially at higher levels: induction CYP 1A2
- What about liver samples from Curacao?

Levels in livers (lipid based)



Levels in livers (fresh weight)



ML sum-TEQ: 0.5 pigs/cows, 2.0 sheep

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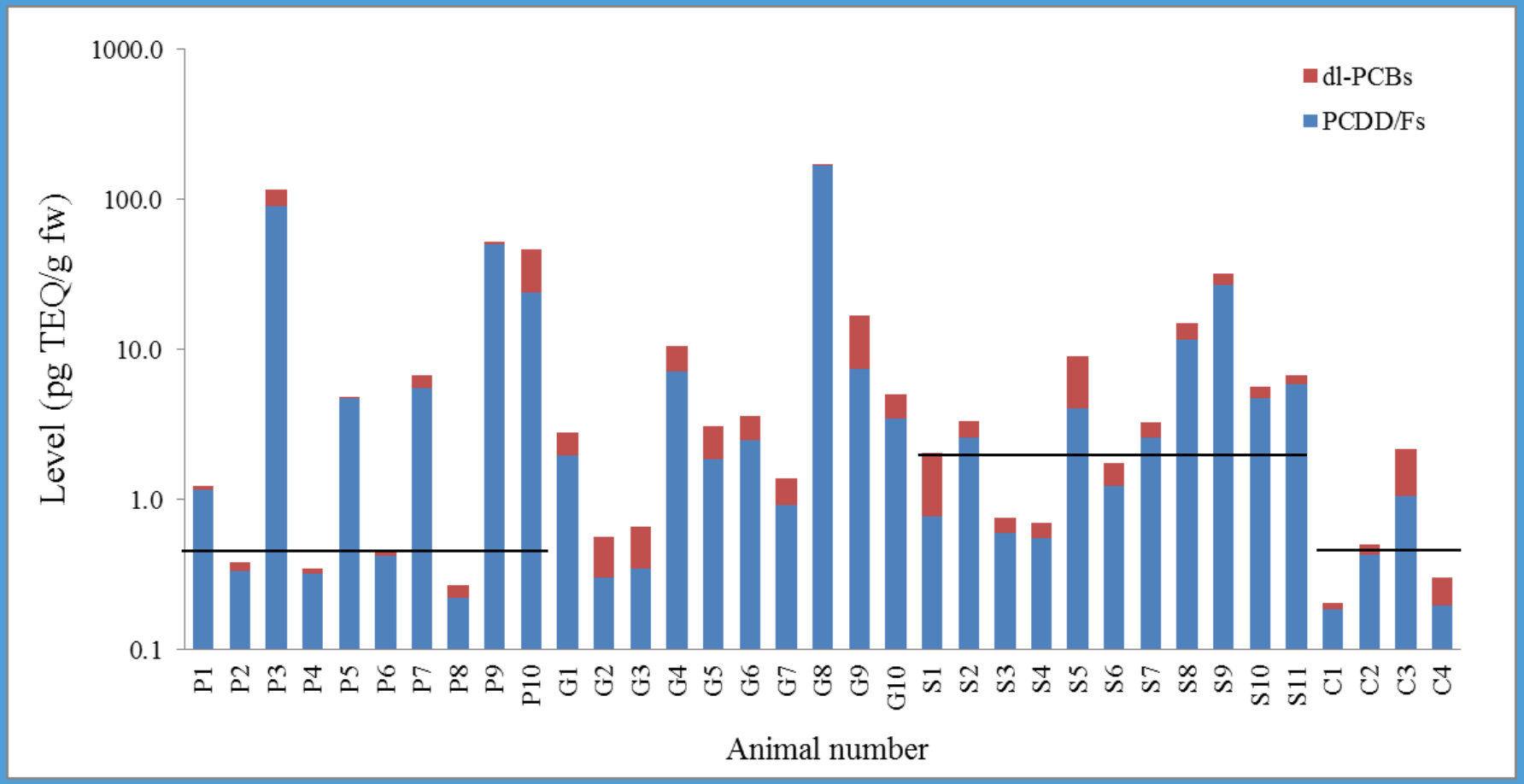
Levels in livers (fresh weight)



ML sum-TEQ: 0.5 pigs/cows, 2.0 sheep

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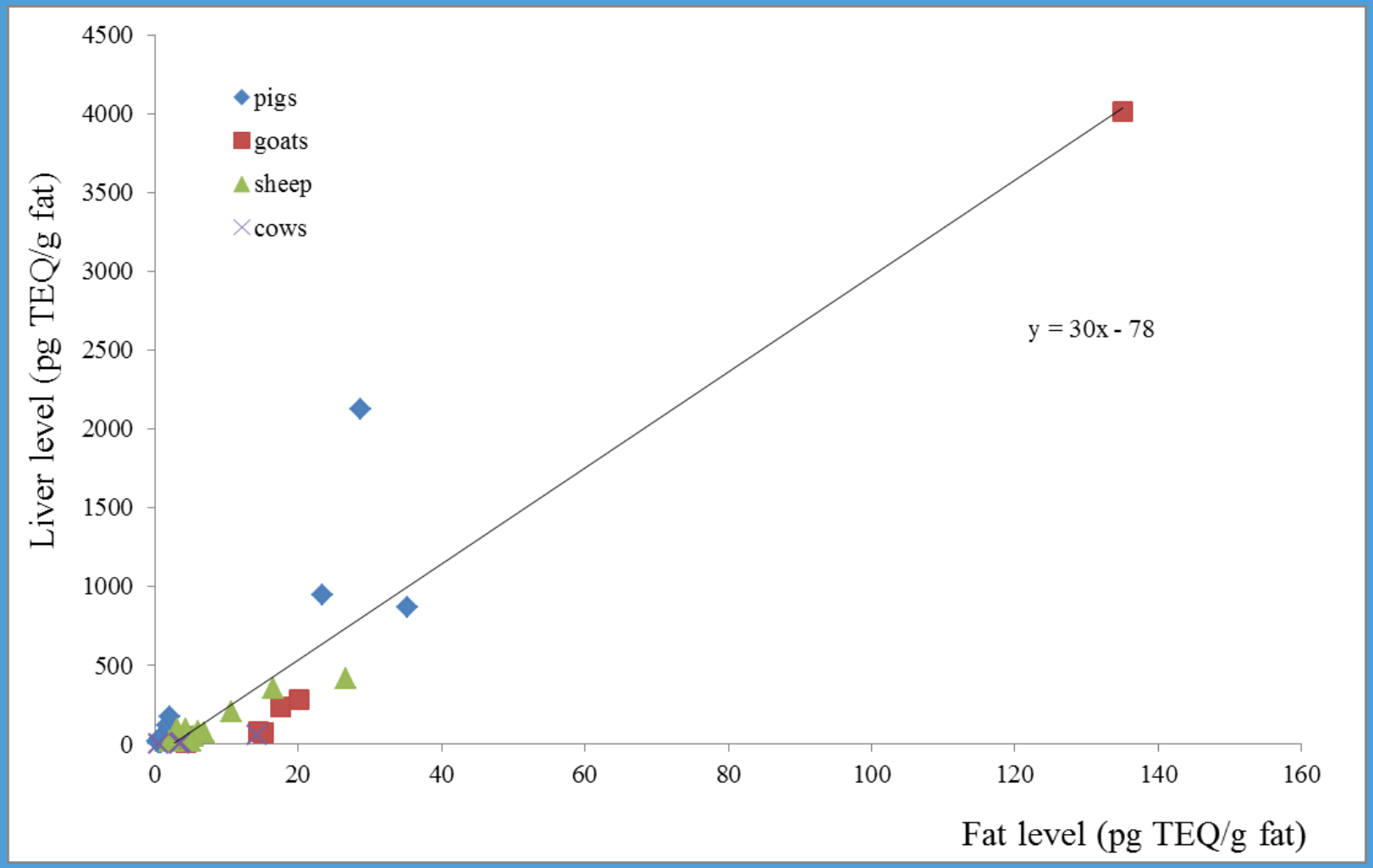
Relation fat/livers



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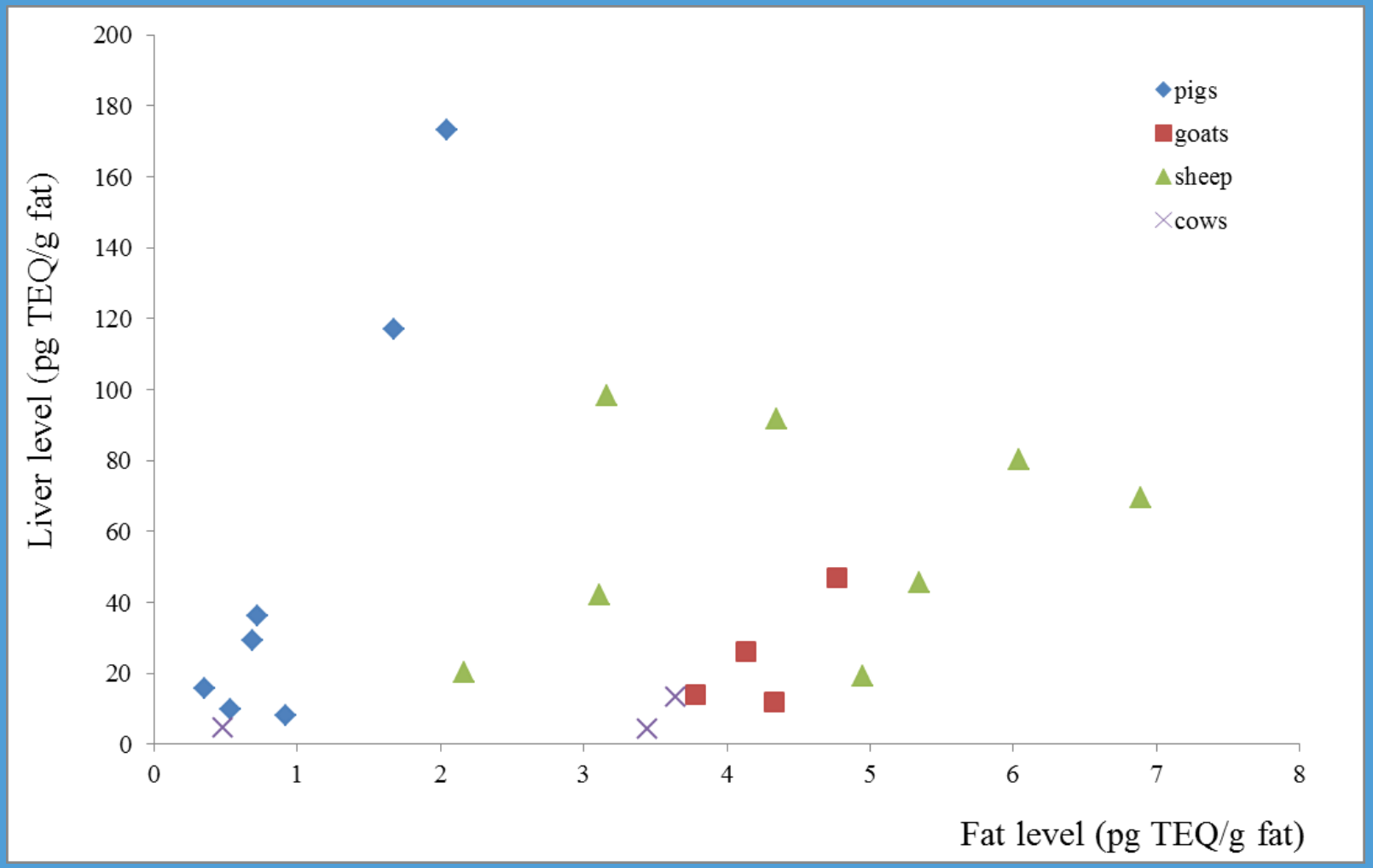
What about low exposure?



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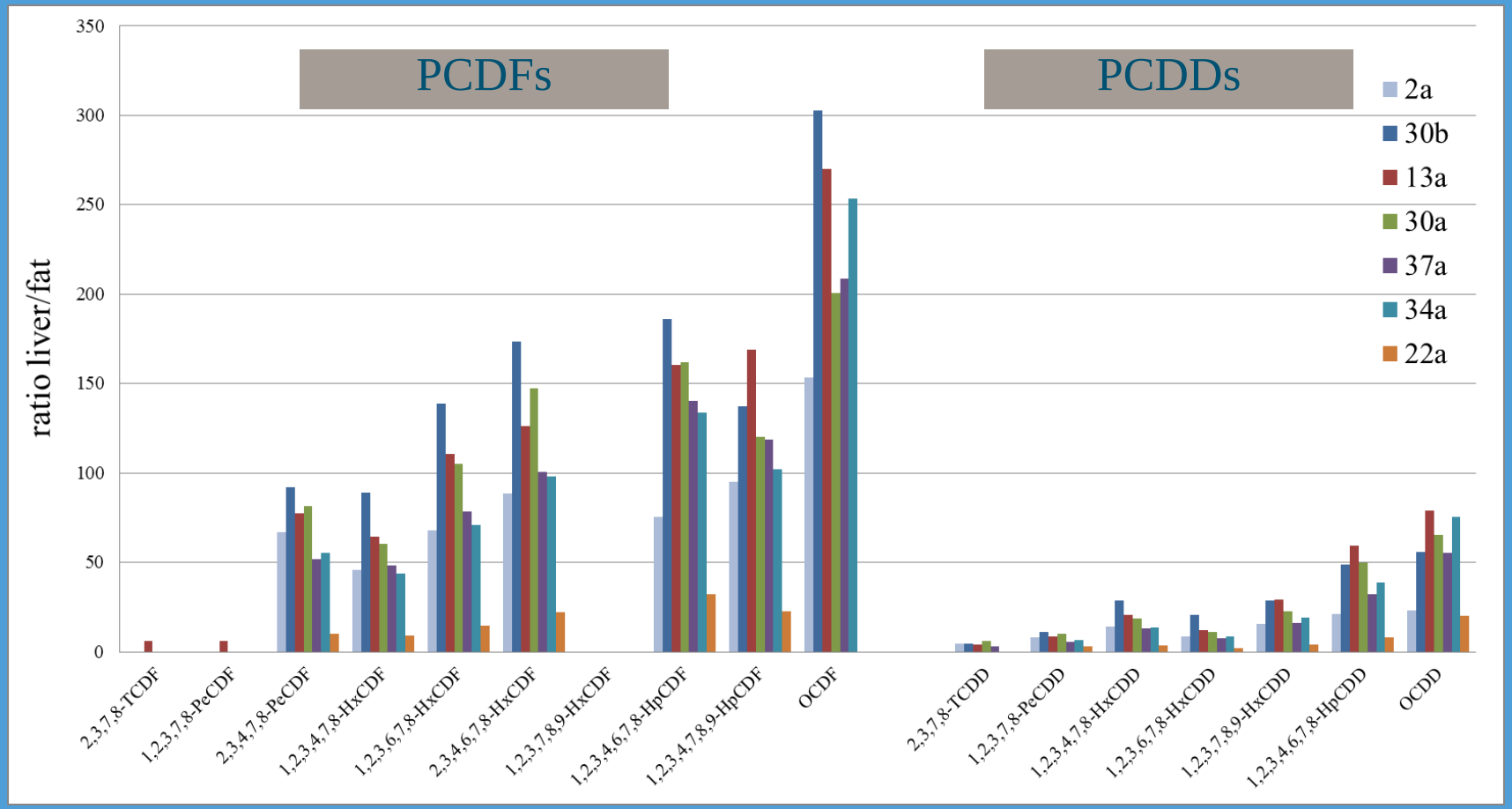
Ratio liver/adipose tissue



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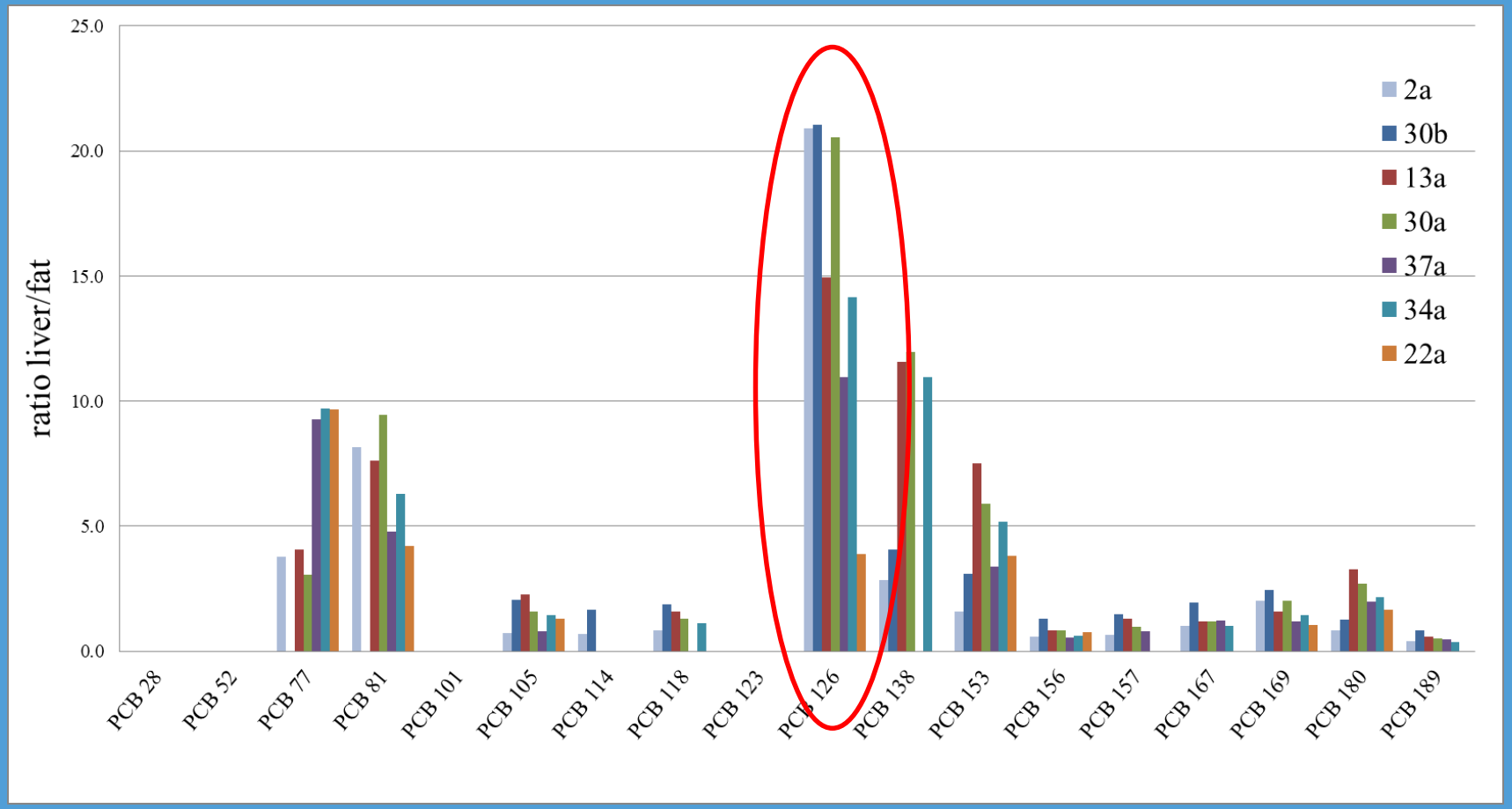
Ratio liver/adipose tissue



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General conclusions

- Current limits in feed too high to prevent non-compliant levels in eggs
  - Product Board eggs uses lower “guidance value”
- In lactating cows borderline
- Low levels in soil (few ng TEQ/kg) may cause problems in laying hens and maybe other species
  - Only estimates about soil intake
  - Current soil limits too high

Thank you for your attention

Questions?

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