

Dioxins analysis at RIKILT

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CONTENT

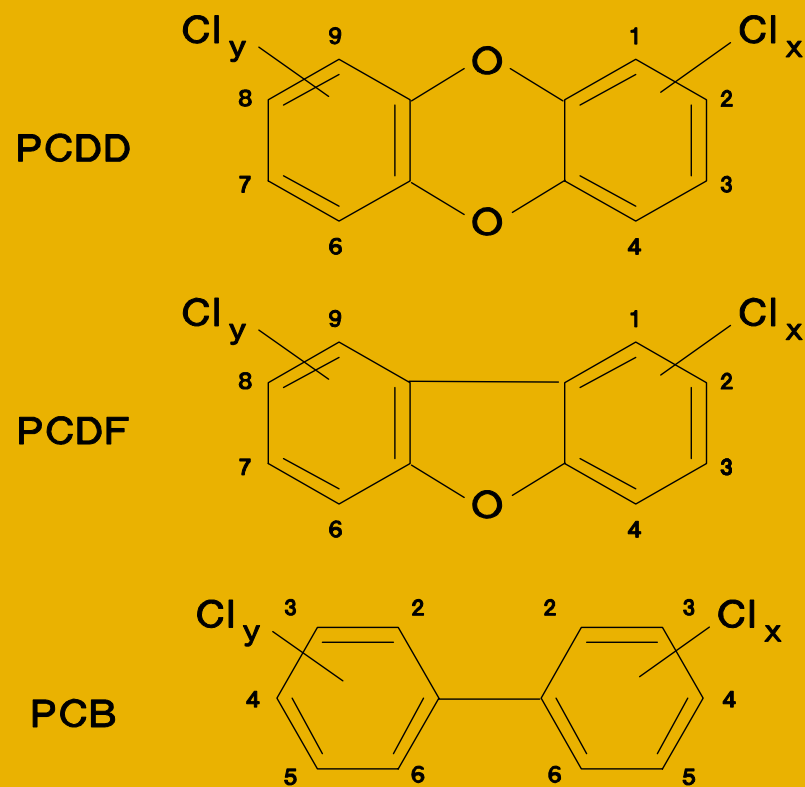
- Introduction on dioxins
- Use of CALUX and GC-HRMS
- Recent affaires
- RIKILT approach and the next generation of GC-HRMS method



Dioxins in milk (1989)



Dioxins and PCBs



Dioxin analysis

Sophisticated analytical methods required:

- Extraction of dioxins from fat (acid/base silica, GPC)
- Removal of possible interfering contaminants (Al_2O_3)
- Separation dioxins and PCBs on activated carbon column
- Separation and confirmation of different congeners (HRGC/HRMS) use of labelled standards for recovery control
- Different analysis of non-ortho and other dioxin-like PCBs



Quantification

Result of dioxin analysis in chicken (content in pg/g fat)

		TEF	
2,3,7,8-TCDF	0,95	0,1000	0,09
1,2,3,7,8-PeCDF	<0,10	0,0500	<
2,3,4,7,8-PeCDF	0,30	0,5000	0,15
1,2,3,4,7,8-HxCDF	0,22	0,1000	0,02
1,2,3,6,7,8-HxCDF	0,14	0,1000	0,01
2,3,4,6,7,8-HxCDF	0,19	0,1000	0,02
1,2,3,7,8,9-HxCDF	<0,10	0,1000	<
1,2,3,4,6,7,8-HpCDF	0,98	0,0100	0,01
1,2,3,4,7,8,9-HpCDF	<0,25	0,0100	<
OCDF	1,20	0,0001	0,00
2,3,7,8-TCDD	0,57	1,0000	0,57
1,2,3,7,8-PeCDD	0,70	1,0000	0,70
1,2,3,4,7,8-HxCDD	0,37	0,1000	0,04
1,2,3,6,7,8-HxCDD	0,79	0,1000	0,08
1,2,3,7,8,9-HxCDD	0,99	0,1000	0,10
1,2,3,4,6,7,8-HpCDD	4,78	0,0100	0,05
OCDD	25,40	0,0001	0,00
Total content in pg TEQ/g fat			1,84

Quantification

<i>Congener</i>	<i>Content pg/g</i>	<i>TEF</i>	<i>Content pg TEQ/g</i>
<i>2378-TCDD</i>	0,57	1,0	0,57
<i>123478-HxCDD</i>	0,37	0,1	0,037
<i>OCDD</i>	25,4	0,0001	0,0025
<i>TOTAL</i>			0,61



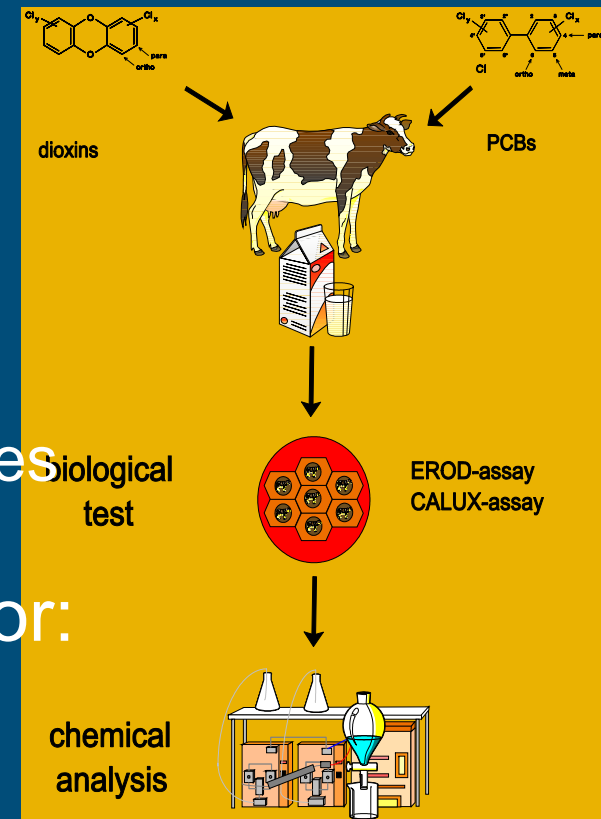
Screening method for dioxins

■ Requirements:

- quick and relatively cheap
- high sample throughput
- obeying the TEQ-principle
- no false-negatives, few false-positives

■ GC/MS reference method used for:

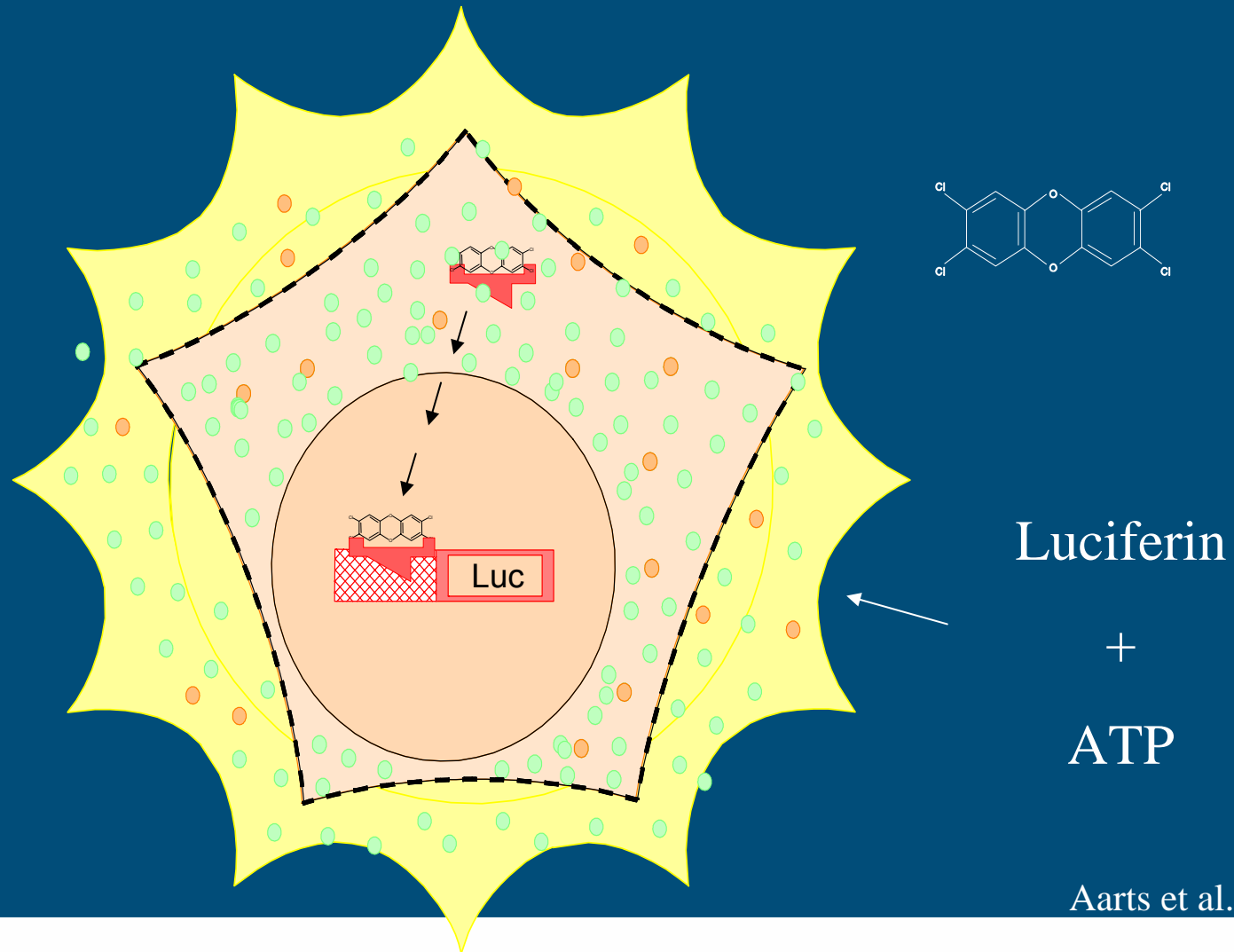
- confirmation
- source identification



CALUX-assay

- Chemical Activated LUCiferase gene eXpression assay
 - Developed by University in Wageningen in cooperation with University of Michigan (later UC Davis) and RIKILT

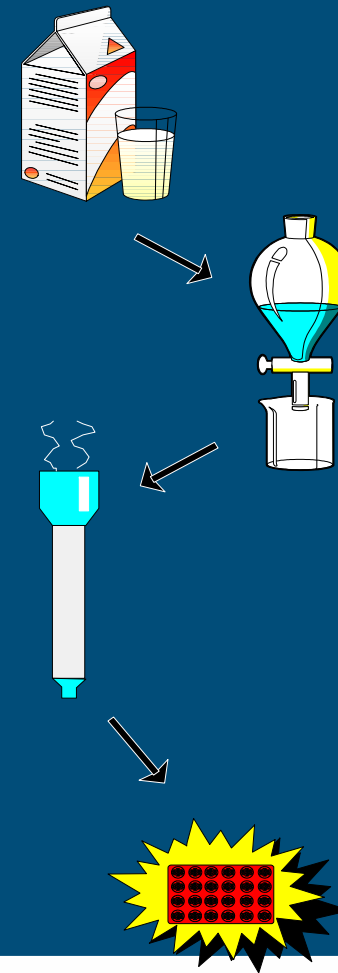
CALUX screening assay



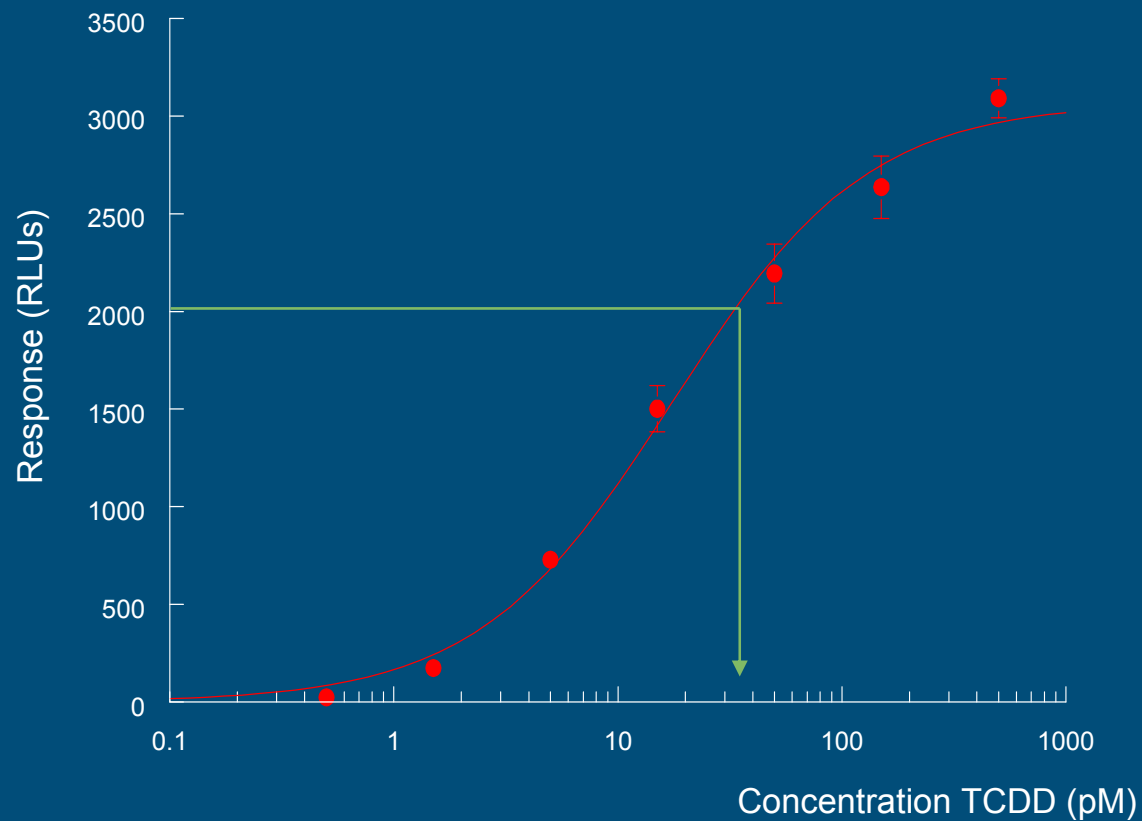
Aarts et al. 1993

Clean-up of samples

- Fat extraction
- Acid silica clean-up (6-8 hrs)
- CALUX-assay (24 hrs incubation)



Quantitative approach



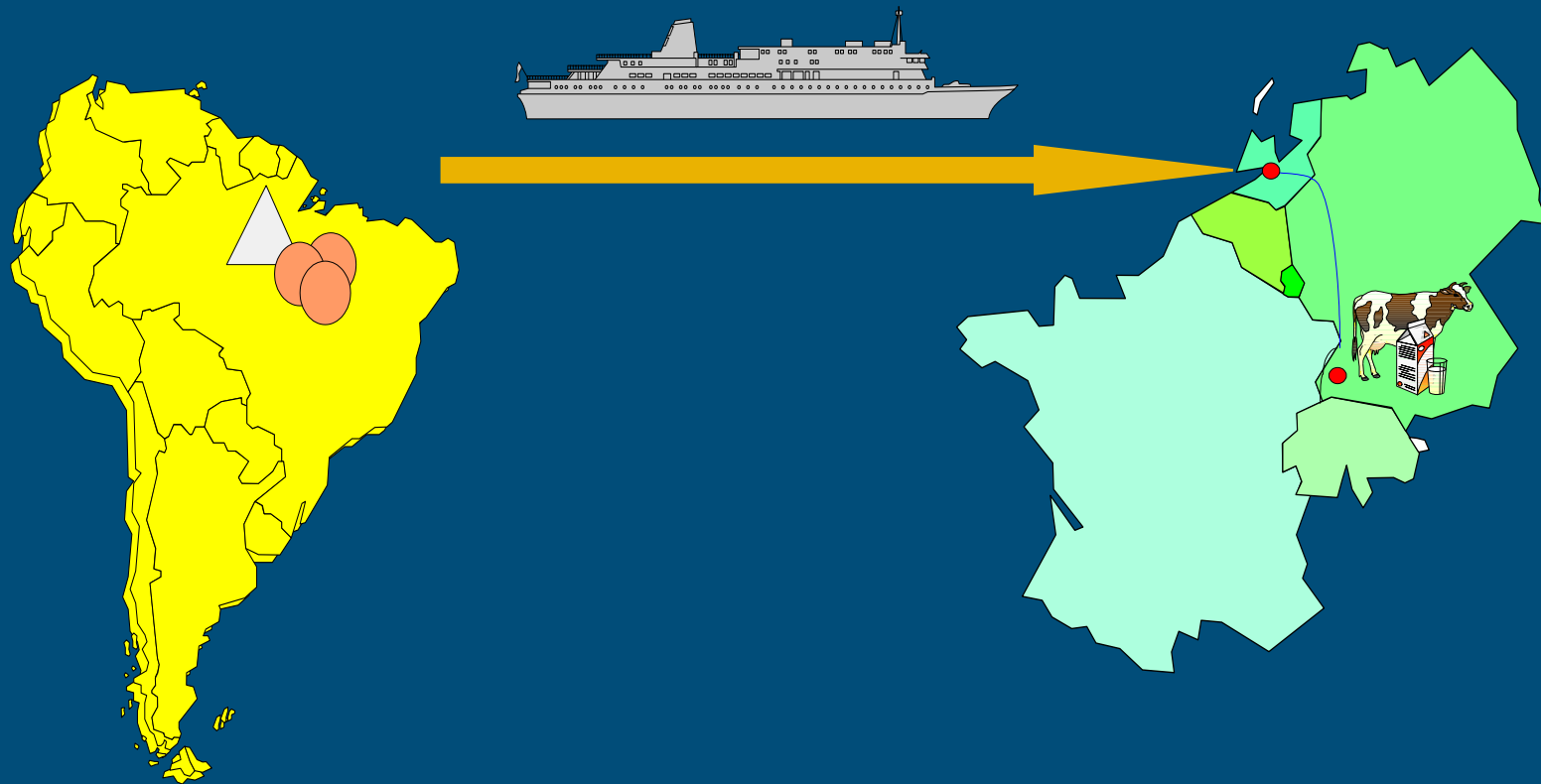
Use of reference samples

- Matrix-specific reference materials are required:
 - To correct for possible contaminants from solvents
 - To correct for possible recovery losses
 - To correct for the difference in “CALUX-TEFs” and WHO-TEFs

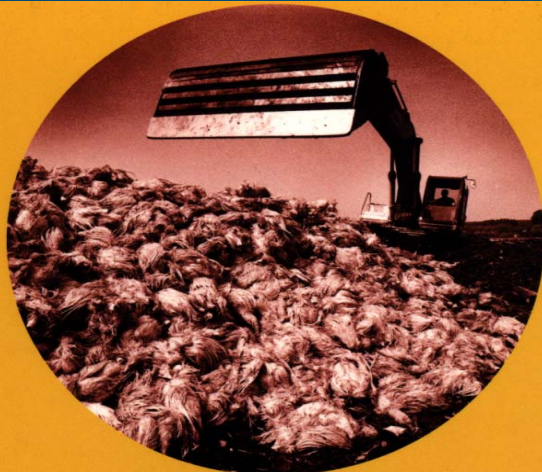
INCIDENTS

- RIKILT has been involved in many incidents on:
 - Dioxins
 - PAH's
 - Hormones
 - Etc

Dioxins in citrus pulp from Brazil (1998)



The Belgian dioxin crisis in 1999



**Vet drijft
altijd boven**

SIEL VAN DER DONCKT

*De verzwegen
dioxinecrisis*



More incidents dioxins in milk (2004)



Elevated dioxin content in milk in 2004

(Lelystad affaire)

- Mixed pooled (RMO) sample of September contains dioxins
(= four RMO's \approx 20 farms) 1,5
pg TEQ/gram fat
- Individual RMO samples analysed using CALUX, one suspected
- Confirmation of suspected sample with GC-HRMS
5,1 pg TEQ/gram fat (three farms)
- Samples of these farms analysed using CALUX
two on background level, one suspected
- GC-HRMS **20 pg TEQ/gram fat**

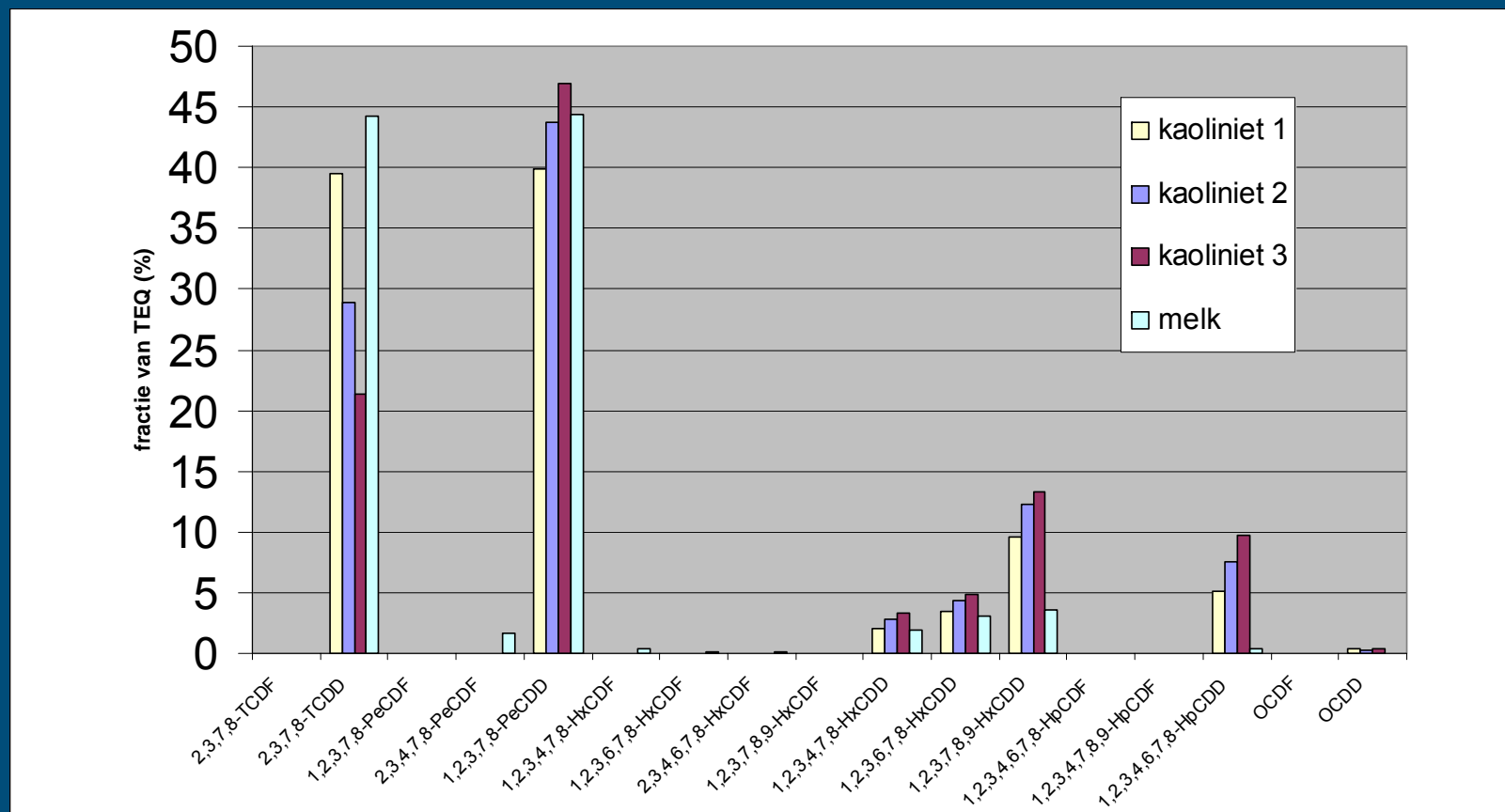


Lelystad affaire

2,3,7,8-TCDF	<0.05
1,2,3,7,8-PeCDF	<0.10
2,3,4,7,8-PeCDF	0,64
1,2,3,4,7,8-HxCDF	0,82
1,2,3,6,7,8-HxCDF	0,35
2,3,4,6,7,8-HxCDF	0,23
1,2,3,7,8,9-HxCDF	<0.10
1,2,3,4,6,7,8-HpCDF	0,3
1,2,3,4,7,8,9-HpCDF	<0.25
OCDF	<0.50
2,3,7,8-TCDD	8,6
1,2,3,7,8-PeCDD	8,62
1,2,3,4,7,8-HxCDD	3,74
1,2,3,6,7,8-HxCDD	5,97
1,2,3,7,8,9-HxCDD	6,94
1,2,3,4,6,7,8-HpCDD	8,27
OCDD	3,59
Total content TEQ[lb]	19,43
Total content TEQ [ub]	19,45



Pattern in milk resembles kaolinic clay (1999)



Samples from contaminated farm

- All feeding stuffs sampled (≈ 25)
- No kaolinic clay present on farm
- Several samples suspected using CALUX, only **potato peels** highly contaminated, level in milk can be explained
- CALUX result confirmed using GC/HRMS; pattern comparable with milk



Potato peels





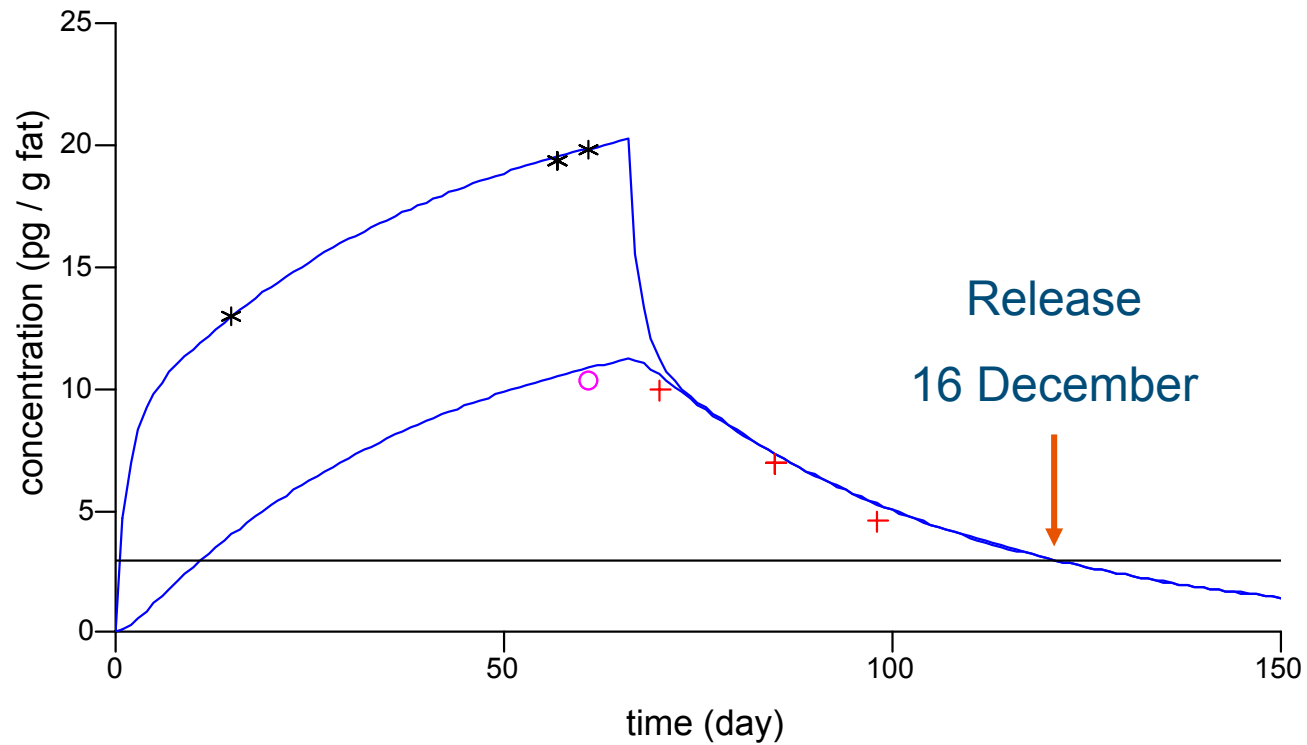
source ?????



Source ??

- Hint from AID: Since the summer of 2004 McCain uses a different procedure for selection of the potatoes
- Clay instead of salt
- Samples clay taken
- Content 1600 ng TEQ/kg
- Similar pattern as potatoes

Contamination of farm in Lelystad in time



Model RIVM



- More affaires in 2004 ????????????



Samples from Lickebaert-area (near Rotterdam)

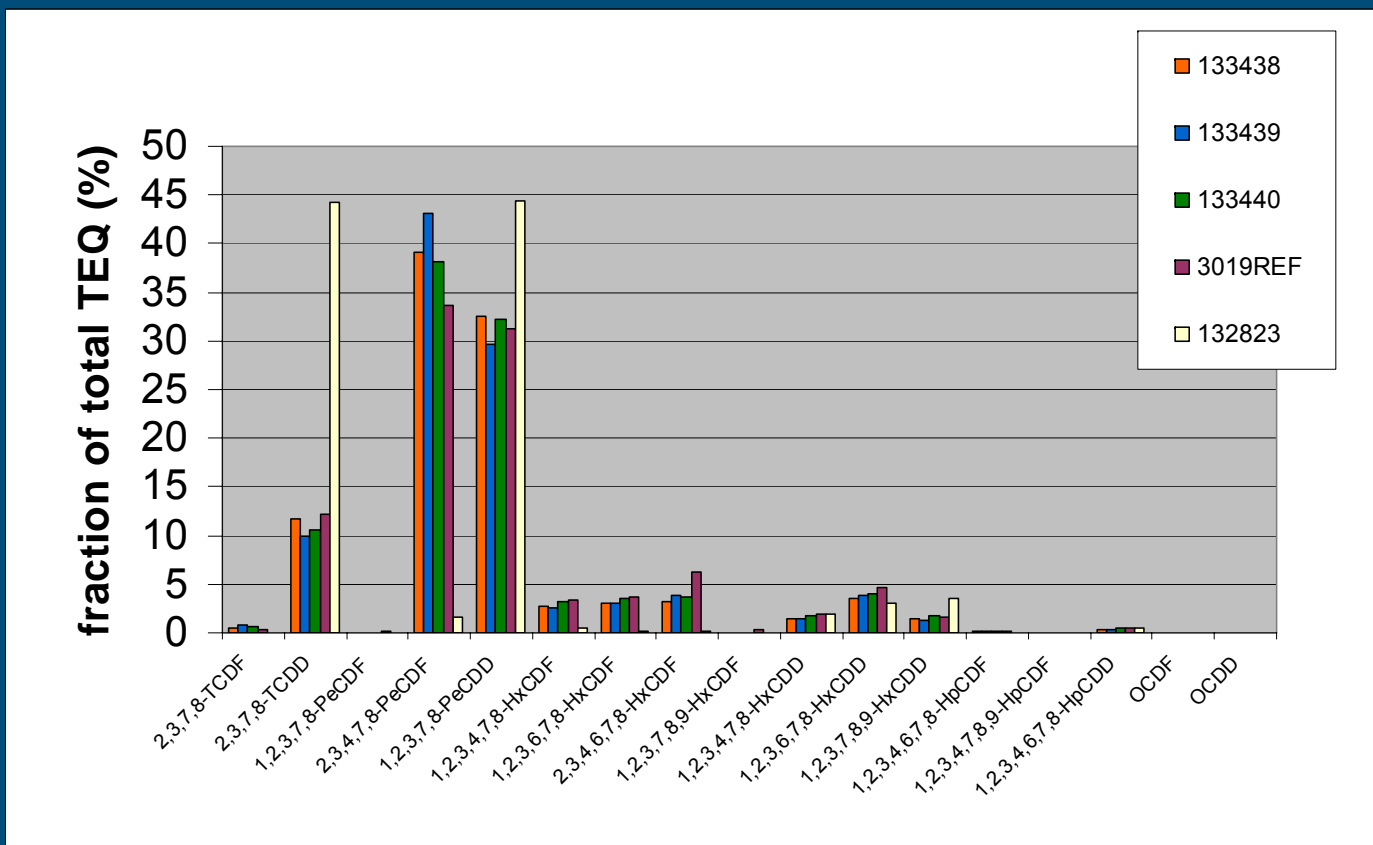
- A number of contaminated milk samples (4-5 pg TEQ/g fat)
- Pattern comparable with reference sample milk from the same area as in 1989
- Extended screening of milk samples with CALUX
- Last quarter of 2004 levels are decreasing



Lickebaert affaire



Dioxin pattern in milk samples from Lickebaert



RIKILT APPROACH FOR DIOXIN

ANALYSIS (1)

- Screening all samples using CALUX
- Compare CALUX response of samples with appropriate reference samples (tested with GC-HRMS)
- All suspected samples have to be confirmed with GC-HRMS
- 5-10 % of negative samples have to be analysed with GC-HRMS
- New matrices always have to be analysed with both techniques



RIKILT APPROACH FOR DIOXIN

ANALYSIS (2)

- GC-HRMS method is currently modified by:
 - Extraction using ASE
 - Animal feed with toluene after pre-wetting
 - Biological samples after freeze drying with hexane
 - Purification using power prep
 - Combination of four columns
 - Jumbo acidified silica column (capacity 3 gram fat)
 - Silica column
 - Al₂O₃ column
 - Carbon column



RIKILT APPROACH FOR DIOXIN

ANALYSIS (3)

- Next steps
- After power prep clean-up two fractions are obtained
 - Fraction A contains mono-ortho + indicator pcb's
 - Fraction B contains dioxins + non-ortho pcb's
 - Both fractions are automatically concentrated using turbovap (keeper is added) ; Endpoint + fixed time
- Concentrated fractions are analysed on two GC-HRMS
 - Fraction A 2 µl using splitless injection
 - Fraction B 25-50 µl using LVI



RIKILT APPROACH FOR DIOXIN ANALYSIS (3)

- Next steps
- Positive samples are analysed by TOF-MS
- In 2005 GC X GC will be implemented

