

8th BioDetectors 2014

"DIOXINS/PCBs, ENDOCRINE DISRUPTERS (EDC), OBESOGENS AND EMERGING POLLUTANTS"

Turin, 26 september 2014

DR-CALUX bioassay as screening method for the detection of contamination by dioxins in Piedmont region

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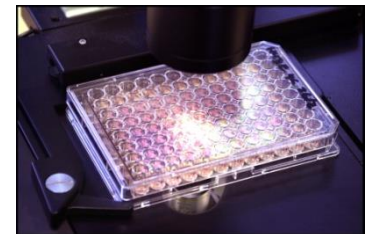
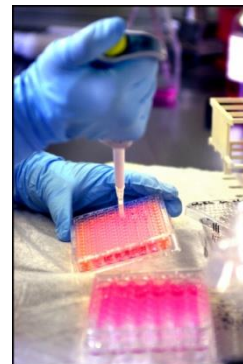
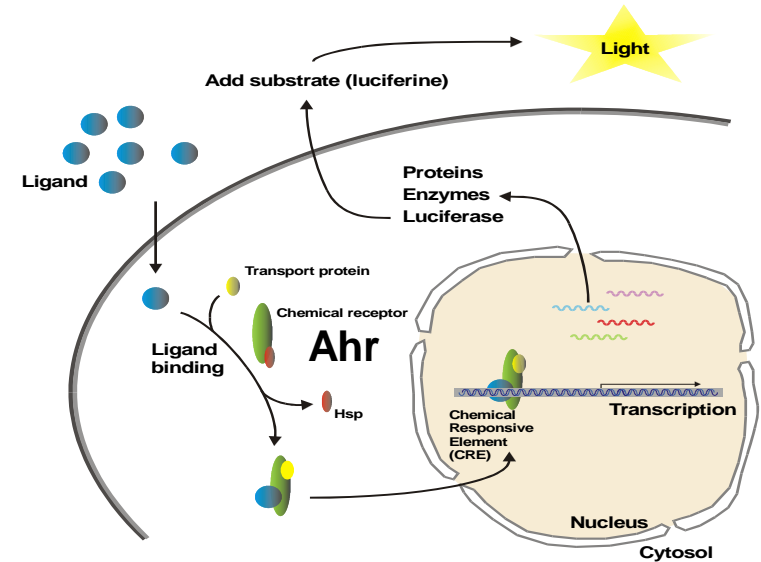
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DR-CALUX in IZSTO since may 2011

Main activities:

- ✓ Research projects
- ✓ Biomonitoring of risk areas



RESEARCH PROJECTS by DR CALUX (1) 2010/2011

“To analyze milk coming from Piedmont farms, by screening method for dioxin and PCBs detection ”

Co-founding by IZSPLV + Fondazione CRT (Cassa di Risparmio di Torino);

Aim of the **starting project**:

- 1) to evaluate the BDS DR CALUX[®] method as a screening tool for monitoring dioxin/PCBs in food, particularly in cow milk coming from Piedmont region;
- 2) to evaluate if this method is really applicable in our labs;
- 3) to evaluate the ICC value of the BDS DR CALUX[®] vs the HRGC/HRMS



30 MI

| ID | RISULTATO HRGC/HRMS pg/g grasso | RISULTATO DR-CALUX® pg/g grasso | DEV. ST. (±) DR- CALUX® |
|----------------|------------------------------------|------------------------------------|----------------------------|
| 6-Val di Susa | 8,5 | 11 | 0,31 |
| 13-Val di Susa | 2,7 | 2,7 | 0,11 |
| 14-Val di Susa | 4 | 3,3 | 0,18 |
| 15-Val di Susa | 7,4 | 3,9 | 0,077 |
| 20-Val di Susa | 3,8 | 6,8 | 0,22 |
| 21-Val di Susa | 2,4 | 2,7 | 0,099 |
| 23-Val di Susa | 7,9 | 13 | 0,77 |
| 31-Val di Susa | 14,61 | 15 | 0,46 |
| 35-Val di Susa | 5,93 | 4,1 | 0,21 |
| 40-Val di Susa | 4,75 | 4,2 | 0,38 |
| 41-Val di Susa | 6,45 | 8,2 | 0,23 |
| 46-Val di Susa | 8,26 | 9 | 0,65 |
| 52-Val di Susa | 4,39 | 3,8 | 0,17 |

LIANT

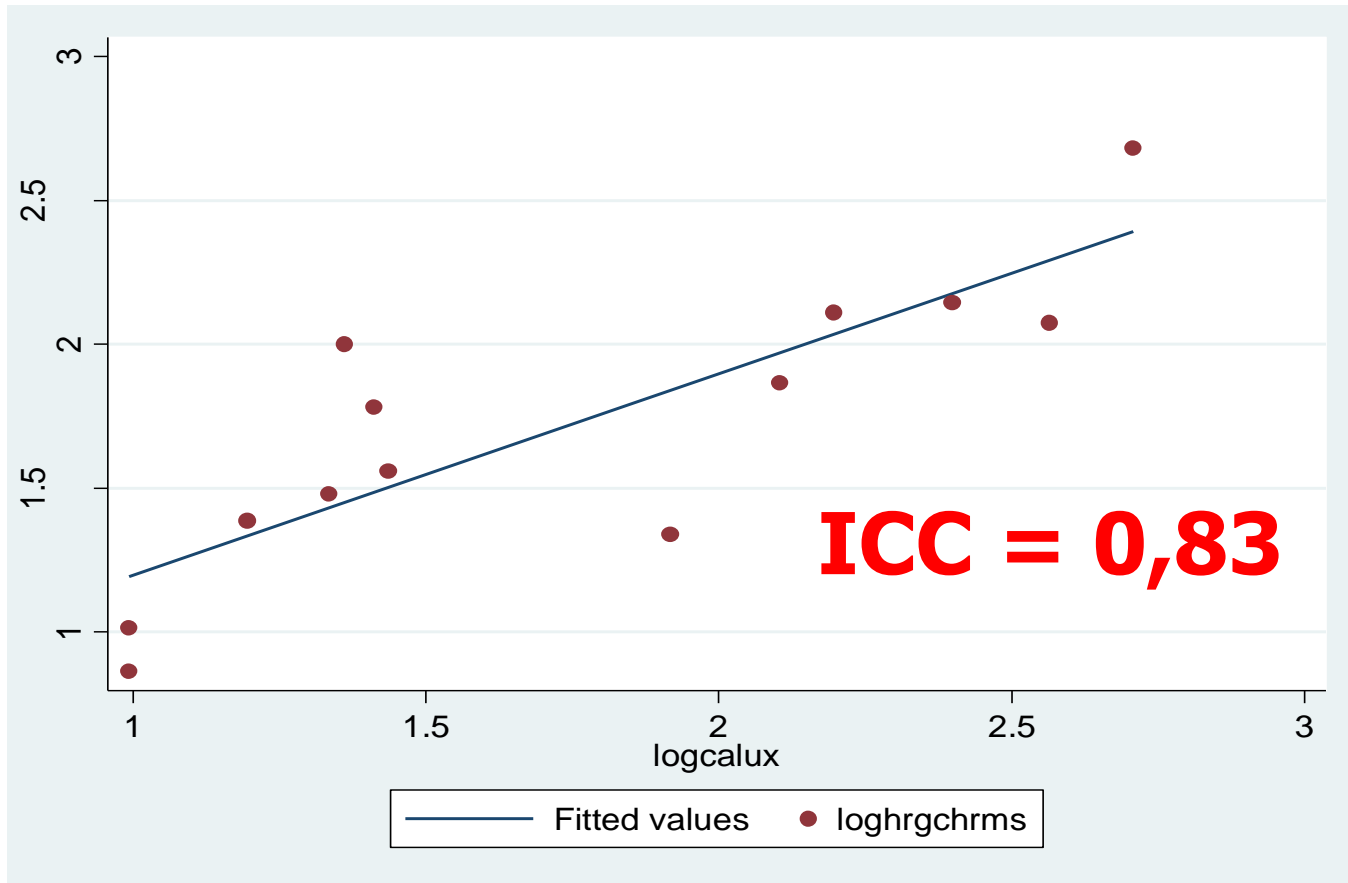


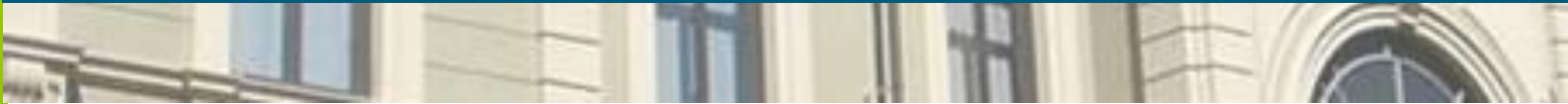
**13 R
REGIO**

EDMONT

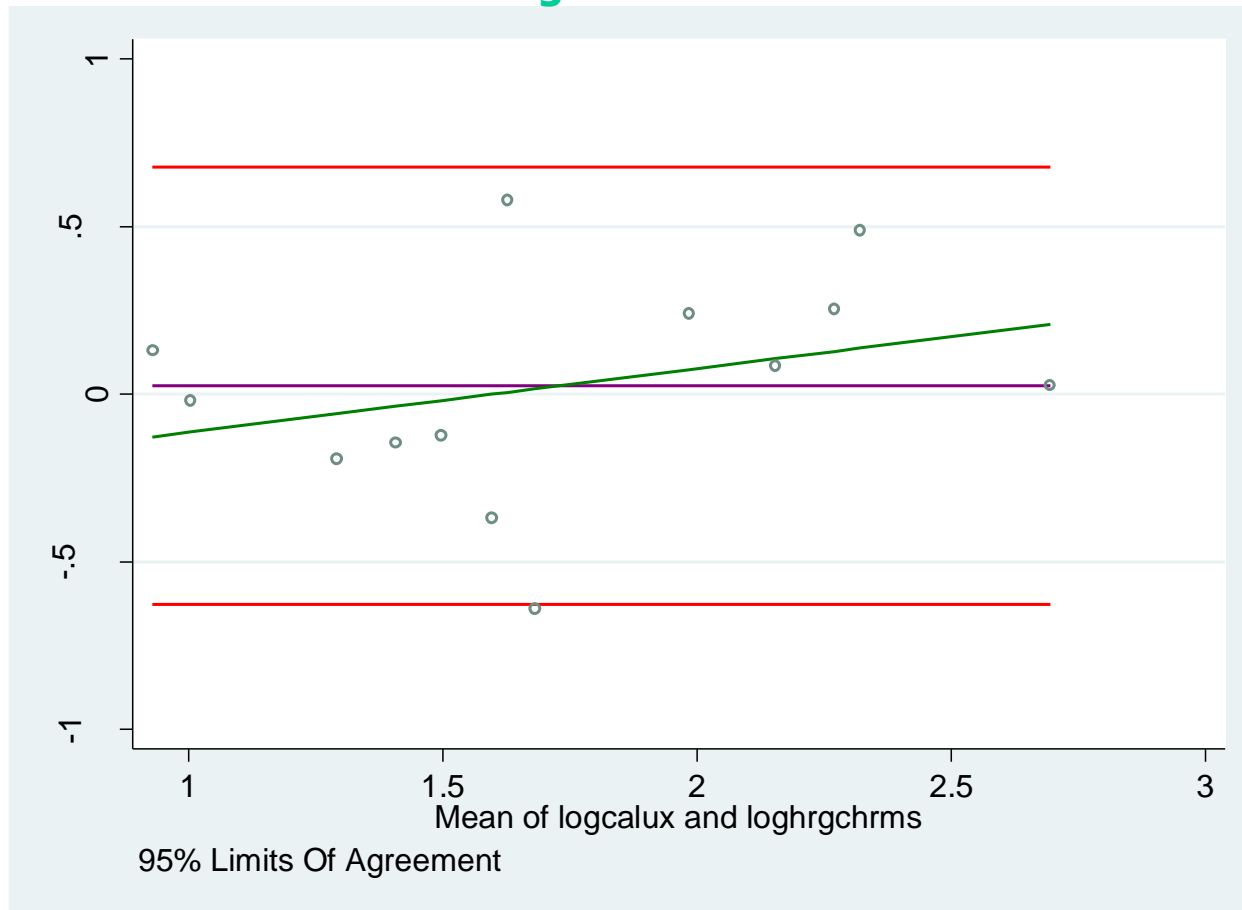


**Scatter plot of the logarithm values obtained by Calux
versus the ones obtained by HRGC/HRMS,
In comparison with the fitted values**





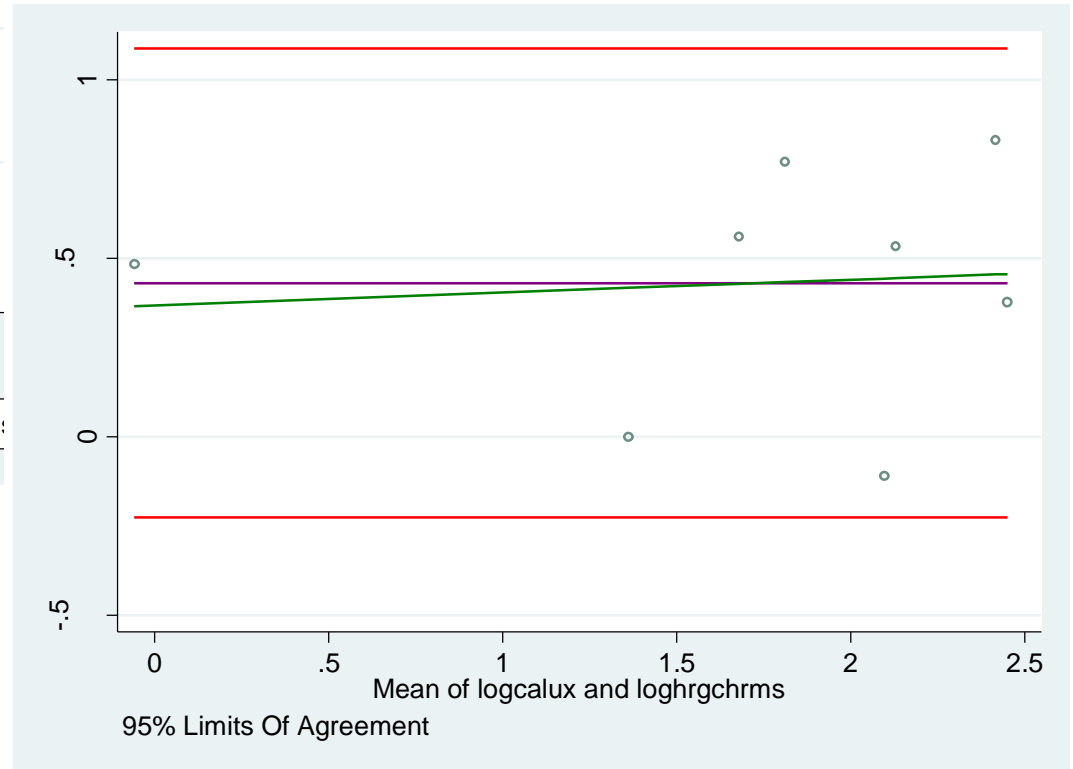
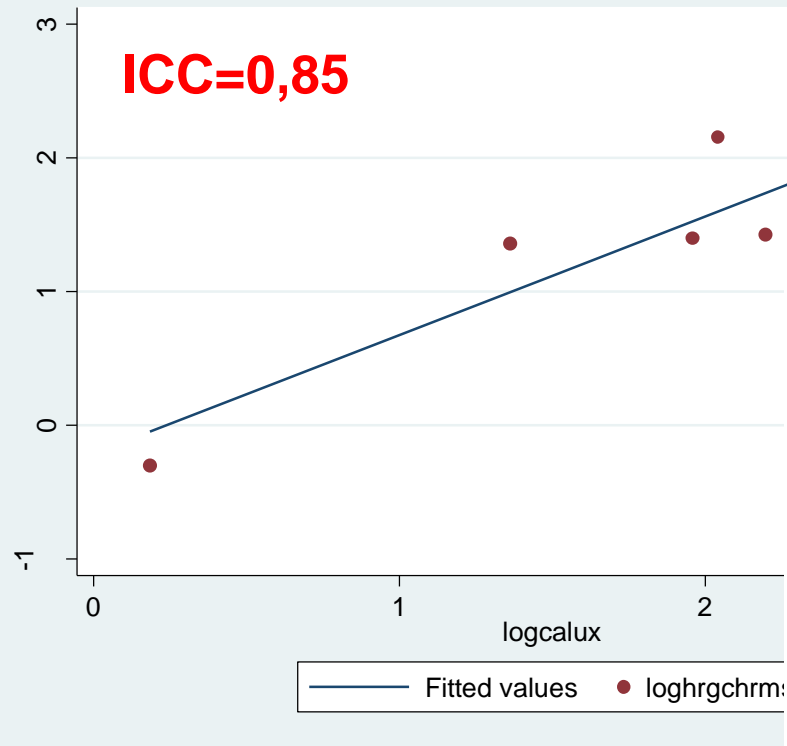
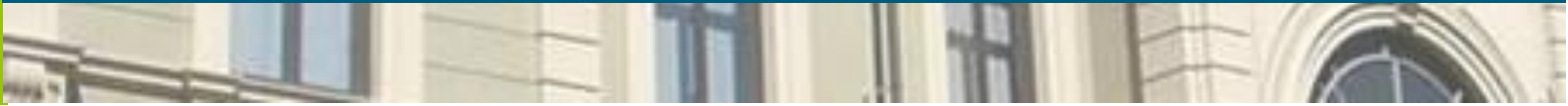
Bland Altman plot of milk log values:
it represents differences for coupled values in comparison with a mean value
Violet line: the ideal condition in which the variance between the two groups is similar
Green line: the regression line from the data



... and the eggs?

| ID | HRGC/HRMS sum PCDD/F+dl- PCB | DR-CALUX [®] sum PCDD/F+dl-PCB | Dev. st. DR- CALUX [®] |
|-----|------------------------------------|--|------------------------------------|
| U7 | 62 | 27 | 0,14 |
| U8 | 3,9 | 3,9 | 0,25 |
| U9 | 8,6 | 7,7 | 0,23 |
| U10 | 6,45 | 11 | 1,40 |
| U12 | 9,6 | 14 | 0,53 |
| U13 | 4,17 | 9 | 0,10 |
| U14 | 4,05 | 7,09 | 0,09 |
| U15 | 7,4 | 17 | 0,21 |
| U16 | 0,74 | 1,2 | 0,09 |





OBSERVED VARIABILITY

mainly depends by the variance between the subjects (milk or egg samples),
but not by the variance into the subjects



There is no significant difference between the methods
affecting the obtained values of the analyzed samples

Intraclass Correlation Coefficient (ICC)

shows a good agreement between the Calux and HRGC/HRMS values

Anyways, due to the particular characteristics of the examined samples (mostly dl-PCB)

we should to:

- 1) Evaluate the results once divided by group of contaminants (PCDD/F and dl-PCB) – florasil columns
- 2) Re-test the method increasing the Nr. of the samples especially with compliant samples
- 3) Re-evaluate the data on BEQ basis (since Reg UE 252/2012 21/03/2012)

RESEARCH PROJECTS by DR CALUX (2) 2011/2014

"To develop screening biomolecular techniques for the detection of the dioxin and dioxin like contaminants exposure in cows"

Founding: European/Regional Founding Program (POR-FESR 2007-2013)

Matrices examined by DR-CALUX: bovine blood serum; bovine milk

1. IRTA S. r.l.
2. Università di Torino – Dip. di Patologia Animale
3. Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta (IZSPLVA)
4. Usseglio Nanot Paolo
5. CORI S.r.l.

3 SMI

2 OR

Aim of the project:

To develop a screening system alternative to the official ones, cheaper and faster



HUMAN BLOOD SERUM by DR CALUX

Environmental Health



Talanta 85 (2011) 2484–2491

Research
AhR transcriptional activity in serum of Inuits across Greenlandic districts
 Manhai Long¹, Bente Deutch² and Eva C Bonefeld-Jorgensen^{*1}

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Talanta

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Published: 23 October 2007

Environmental Health 2007, 6:32 doi:10.1186/1476-069X-6-32

Quantification of PCDD/Fs and dioxin-like PCBs in small amounts of human serum using the sensitive H1L7.5c1 mouse hepatoma cell line: Optimization and analysis of human serum samples from adolescents of the Flemish human biomonitoring program FLEHS II

K. Croes^{a,h,*}, K. Van Langenhove^a, E. Den Hond^b, L. Bruckers^c, A. Colles^b, G. Koppen^b, I. Loots^d, V. Nelen^e, G. Schoeters^b, T. Nawrot^{f,g}, N. Van Larebeke^h, M.S. Denisonⁱ, T. Vandermarken^a, M. Elshout^a, W. Brouwer^a

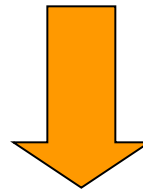
Table 6
 Literature overview for the PCDD/F concentrations from human biomonitoring studies in different countries.

| Reference | Country | Period | Population | N | Calculation | Unit | Value | Method |
|-----------------------|-------------------|-----------|--|------|------------------|--------------------|---------------------------------|----------------------------------|
| This study | Flanders | 2008–2009 | Students (14–15 years old), general population | 173 | GM (95%CI) | pg CALUX-BEQ/g fat | 108 (101–114) | UDC-CALUX, H1L7.5c1 PCDD/F |
| | Flanders | 2008–2009 | Students (14–15 years old), general population | 172 | GM (95%CI) | pg CALUX-BEQ/g fat | 32.1 (30.1–34.2) | UDC-CALUX H1L7.5c1, dl-PCB |
| Van Wouwe et al. [13] | Belgium | 2000 | Adults, men and women | 341 | GM | pg CALUX TEQ/g fat | 41.8 | XDS-CALUX, H1L6.1c2 PCDD/F |
| | Belgium | 2000 | Adults, men and women | 341 | GM | pg WHO-TEQ/g fat | 25.7 | GC-HRMS, PCDD/F |
| Long et al. [1] | Greenland | 2002–2004 | Adults, men | 75 | Median | pg CALUX TEQ/g fat | 197 | UDC-CALUX, Hepa1.12cR |
| | Poland | 2002 | Adults, men | 99 | Median | pg CALUX TEQ/g fat | 312 | UDC-CALUX, Hepa1.12cR |
| | Sweden | 2002 | Adults, men | 78 | Median | pg CALUX TEQ/g fat | 428 | UDC-CALUX, Hepa1.12cR |
| | Ukraine | 2002 | Adults, men | 86 | Median | pg CALUX TEQ/g fat | 337 | UDC-CALUX, Hepa1.12cR |
| Koppen et al. [22] | Flanders, Peer | 1999 | Adults, women, 50–65 years old | 22 | Mean (SD) | pg CALUX TEQ/g fat | 37.2 (13.1) | BDS-CALUX, sum PCDD/F and dl-PCB |
| | Flanders, Antwerp | 1999 | Adults, women, 50–65 years old | 25 | Mean (SD) | pg CALUX TEQ/g fat | 35.0 (16.5) | BDS-CALUX, sum PCDD/F and dl-PCB |
| | Flanders, Peer | 1999 | Adults, women, 50–65 years old | 22 | GM (95%CI) | pg WHO-TEQ/g fat | 70.9 (65.3–76.9) | GC-HRMS, sum PCDD/F and dl-PCB |
| | Flanders, Antwerp | 1999 | Adults, women, 50–65 years old | 25 | GM (95%CI) | pg WHO-TEQ/g fat | 78.9 (72.7–85.6) | GC-HRMS, sum PCDD/F and dl-PCB |
| Kayama et al. [23] | Japan | 2002 | Female farmers, 55.5 years old (average) | 1407 | Mean (SD) | pg CALUX TEQ/g fat | 32.3 (12.1) | XDS-CALUX, PCDD/F |
| Todaka et al. [24] | Japan | 2002–2005 | Mothers | 119 | Mean (SD) | pg WHO-TEQ/g fat | 11(4.2) PCDD/F 5.5 (2.5) dl-PCB | GC-HRMS |
| Wittsiepe et al. [25] | Germany | 2000–2003 | Pregnant women, 19–42 years old | 169 | Mean | pg WHO-TEQ/g fat | 167.9 PCDD/F 11.57 dl-PCB | GC-HRMS |
| Burns et al. [26] | Russia | 2003–2005 | Children 8–9 years old | 482 | Median | pg WHO-TEQ/g fat | 21.1 | GC-HRMS |
| Ayotte et al. [27] | Canada | na | Adults, men and women, 25–75 years old | 40 | Median (min–max) | pg CALUX TEQ/g fat | 102 (37–287) | BDS-CALUX, sum PCDD/F and dl-PCB |
| Warner et al. [9] | Italy | 1999 | Women, 20–49 years old | 22 | Mean (min–max) | pg CALUX TEQ/g fat | 30.8 (1.6–67.3) | XDS-CALUX, PCDD/F |

BOVINE BLOOD SERUM by DR CALUX



BioDetection Systems



Pe-BDS-042

Number of Pages: 9

Shake solvent extraction and clean up with two grams of serum for DR CALUX[®] bioassay. 'The one shot approach'

Control value ???

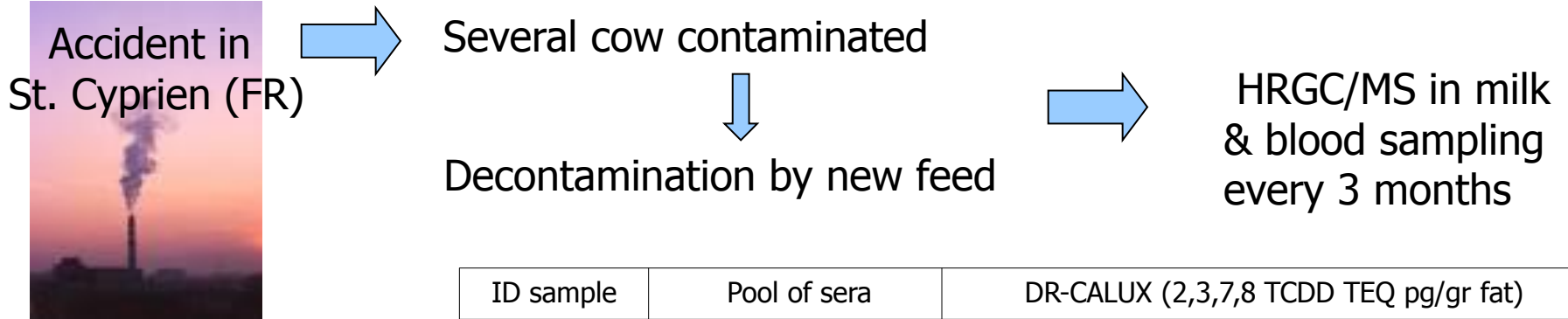


| ID sample | DR-CALUX (2,3,7,8 TCDD TEQ pg/gr fat) |
|-----------|---------------------------------------|
| POOLK1 | 5,9 (±0,18) |
| POOLK2 | 3,5 (±0,18) |
| POOLK3 | 6,4 (±0,29) |

Mean value: 5,27



BOVINE BLOOD SERUM by DR CALUX



T₀

| ID sample | Pool of sera | DR-CALUX (2,3,7,8 TCDD TEQ pg/gr fat) |
|-----------|--------------------|---------------------------------------|
| C1 | POOL 7067/7071 (A) | 110 (±8,3) |
| C2 | POOL 4950/8560 (A) | 100 (±2,6) |

- 1) decontamination of sera directly related to the time
- 2) but not always direct correlation between serum and milk
- 3) DR-Calux in serum possible approach for a non invasive evaluation in non lactating animals

| | | |
|-----|----------|-----------|
| C11 | 8554 (C) | 47 (±4,5) |
| C12 | 8078 (C) | 68 (±6,7) |

RESEARCH PROJECTS by DR CALUX (3) 2013/2015

"Biodetector analysis of the level of dioxin contamination in lake wild and farmed fish species and evaluation of the possible effects on the immune system"

2013 Co- founding: by IZSPLV + Fondazione CRT (Cassa di Risparmio di Torino);

3 Piedmont lakes / supposed different level of contamination / 2 different species *P. fluviatilis* and *R. rutilus* (different level of food chain)

Several aquaculture farms / supposed different level of contamination / *T. iridea*

SS Genetica e immunobiochimica – Calux analyses
SS Ittiopatologia – fishing and necropsies on fish
University of Udine –analyses on fish immune system
SS BEAR – biostatistical analyses



Work in progress

BIO – MONITORING 2012-2014

1) BIC

A

Analyzed
and €

2) BI

Analyze
AIMS

3) E

Analyzed

DI STURA

Interest
bovine milk
IN -> AIM:

PLANT

re area ->
operational

RISIO

the range of
time



Statistical analysis of the data in progress



Acknowledgements

SC Neuroscienze

SS Genetica e Immunobiochimica

Pier Luigi Acutis
Maria Mazza
Simona Sciuto
Luana Dell'Atti

SC Controllo chimico ambientale

SS Laboratorio contaminanti ambientali

Maria Cesarina Abete
Stefania Squadrone
Riccardo Nespoli

SC Epidemiologia e Osservatorio epidemiologico

SS BEAR

Giuseppe Ru
Rosanna Desiato

SS Ittiopatologia

Marino Prearo

