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MILK AND EGGS TESTING FOR DIOXIN-PCB IN LOMBARDIA REGION

Maccabiani Giampietro[1] *, Bertasi Barbara[1], Di Millo Sabrina[1], Tilola Michela[1], Menotta Simonetta[2], Losio Marina Nadia[1], Ferretti Enrica[1]

[1]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, - Via Bianchi 9 – 25124 Brescia - Italy
[2]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, - Via Fiorini 5 – 40127 Bologna – Italy

* e-mail: giampietro.maccabiani@izsler.it

EPIDEMIOLOGICAL STUDIES OF CONTAMINATION IN RAW MILK

STATISTICAL ANALYSIS ON EGGS DATA

Indications that the major source of human background exposure to total PCDD, PCDF, and dl-PCB is food.

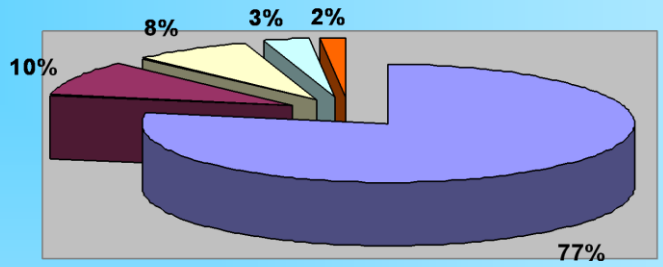
Food of animal origin being the predominant source.

In these last years consumers developed new ideas regarding food products, favouring “natural” and not-treated food, in our region is increased the consumption of the raw milk so as the request of eggs from free range hens.

IZSLER Brescia has decided to apply, as screening test, the DR CALUX® bioassay. The sample exceeding the decided cut off value (2/3 of MRL) have been confirmed by HRGC-HRMS.

The eggs data were compared from a statistical point of view. This comparison is also important to assess the rate of false positives, which could affect the cost-effectiveness of screening test.

In 2010, 325 milk samples (274 raw milk) were analyzed



	D < 2 pg TEQ/g fat	77 %
	2 ≤ D < 2,25 pg TEQ/g fat	10 %
	2,25 ≤ D < 3 pg TEQ/g fat	8 %
	3 ≤ D < 4 pg TEQ/g fat	3 %
	D ≥ 4 pg TEQ/g fat	2 %

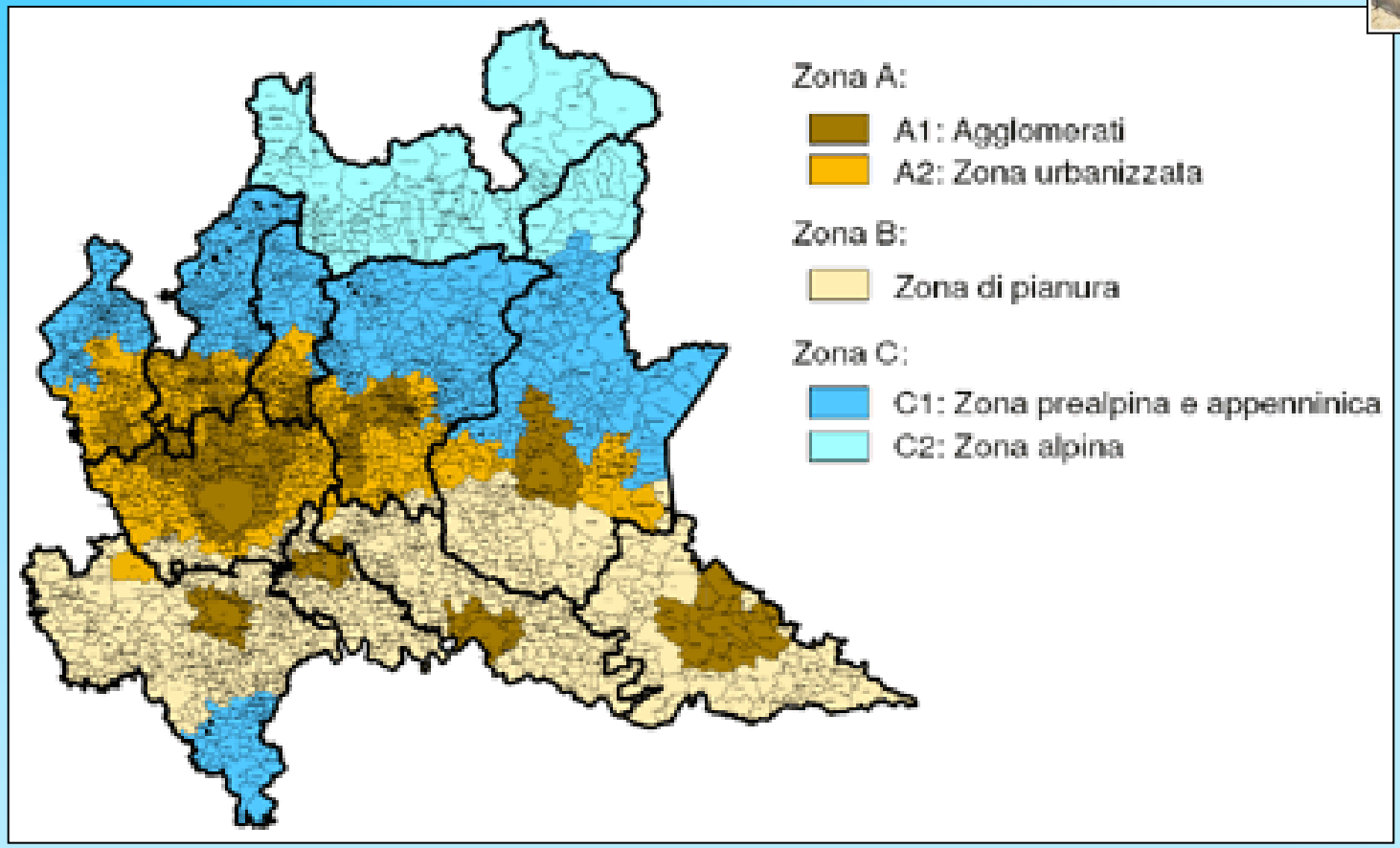
Action level declared 2006/88/CE recommendation

Listed also the samples below the 25% of the maximum level of dioxin contamination, as declared in 2006/1883/CE regulation, modified by 2012/252/CE

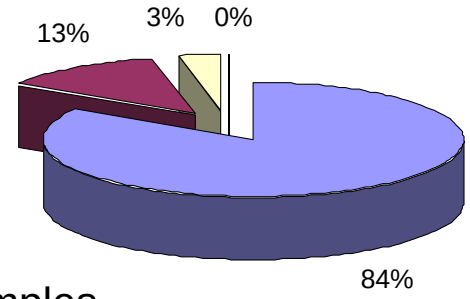
RAW MILK DATA

Samples	D < 2	2 ≤ D < 3	3 ≤ D < 4	D ≥ 4
274	223	36	10	5
Perc. %	81,4	13,1	3,7	1,8

LOMBARDIA REGION

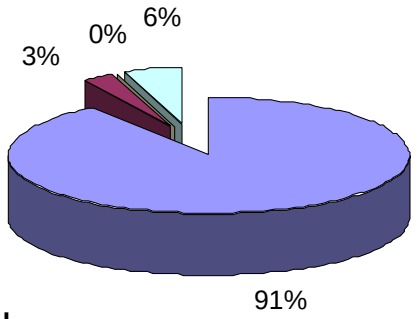


TEQ pg / g fat in raw milk in Bergamo province



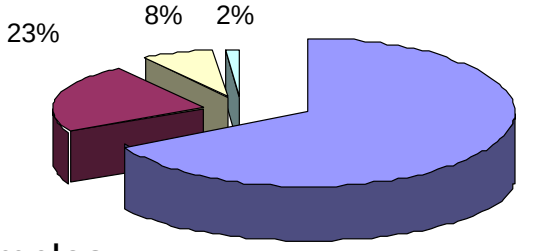
31 samples

TEQ pg / g fat in raw milk in Brescia province



32 samples

Fig.4.3 TEQ pg / g fat in raw milk in Milano province



64 samples

$D < 2$

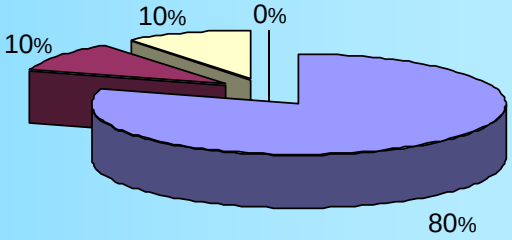
$2 \leq D < 3$

$3 \leq D < 4$

$D \geq 4$

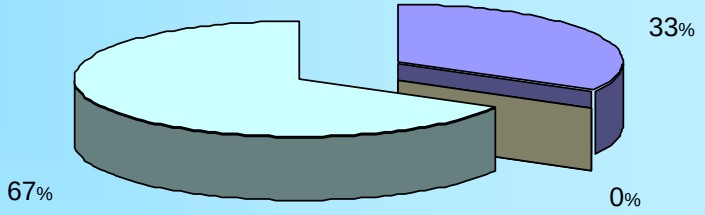
BERGAMO: south-west

TEQ pg / g fat in raw milk in south-west area of Bergamo province



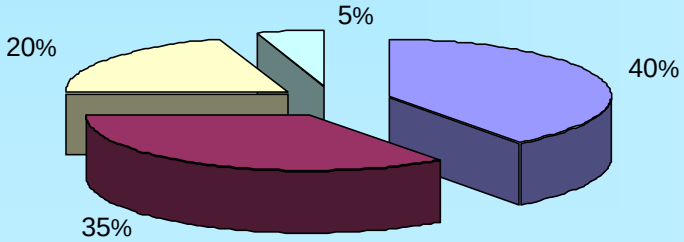
BRESCIA: north-east

TEQ pg / g fat in raw milk in north-east area of Brescia province



MILANO: north-west

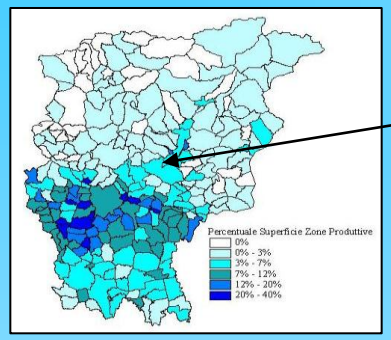
TEQ pg / g fat in raw milk in north-west area of Milano province



- D < 2
- 2 ≤ D < 3
- 3 ≤ D < 4
- D ≥ 4

BERGAMO: south-west

Industrialized area

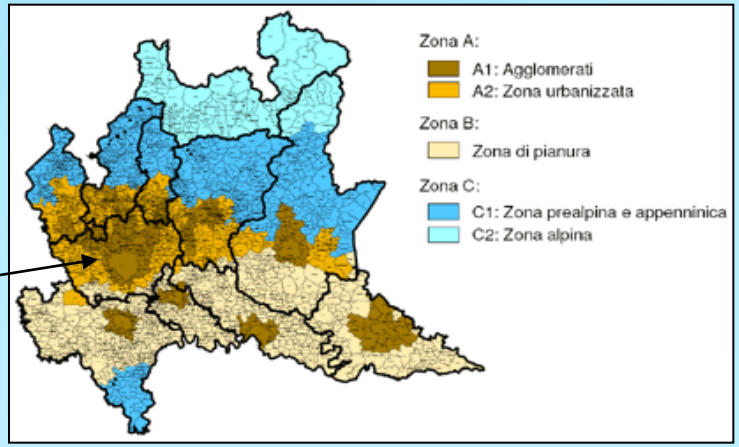


Mountain area

BRESCIA: north-east

MILANO: north-west

Industrialized area



ANALYTICAL RESULTS ON EGGS

EGG SAMPLES					
EU limit (pg TEQ/fat)	DR CALUX (pg TEQ/g fat)	HRGC/HRMS (pg TEQ/g fat)	Cut-Off DR CALUX = Total-TEQ minus 50%	Cut-Off DR CALUX = Total-TEQ minus 25%	Cut-Off DR CALUX = Total-TEQ minus 2/3
6	7,4	5,5	3,0	4,5	4
6	9,1	6,1	3,0	4,5	4
6	23,0	89,6	3,0	4,5	4
6	9,3	13,6	3,0	4,5	4
6	14,5	35,8	3,0	4,5	4
6	29,0	35,1	3,0	4,5	4
6	16,0	10,2	3,0	4,5	4
6	14,0	7,4	3,0	4,5	4
6	11,0	6,9	3,0	4,5	4
6	26,0	26,2	3,0	4,5	4
6	10,0	7,6	3,0	4,5	4
6	18,0	15,9	3,0	4,5	4
6	12,0	12,7	3,0	4,5	4
6	12,0	9,0	3,0	4,5	4
6	12,0	7,6	3,0	4,5	4
6	6,8	3,3	3,0	4,5 (false positive)	4 (false positive)
6	20,0	22,6	3,0	4,5	4
6	13,0	10,4	3,0	4,5	4
6	15,0	14,2	3,0	4,5	4
6	8,5	3,2	3,0	4,5 (false positive)	4 (false positive)
6	7,0	5,9	3,0	4,5	4
6	9,7	5,0	3,0	4,5	4
6	11,0	9,8	3,0	4,5	4
6	7,8	6,1	3,0	4,5	4
6	7,1	2,7	3,0 (false positive)	4,5 (false positive)	4 (false positive)
6	12,0	6,2	3,0	4,5	4
6	9,5	5,0	3,0	4,5	4
6	35,0	61,7	3,0	4,5	4
6	11,0	9,2	3,0	4,5	4
6	15,0	18,7	3,0	4,5	4
6	27,0	38,0	3,0	4,5	4
6	8,2	6,0	3,0	4,5	4
6	18,0	15,5	3,0	4,5	4

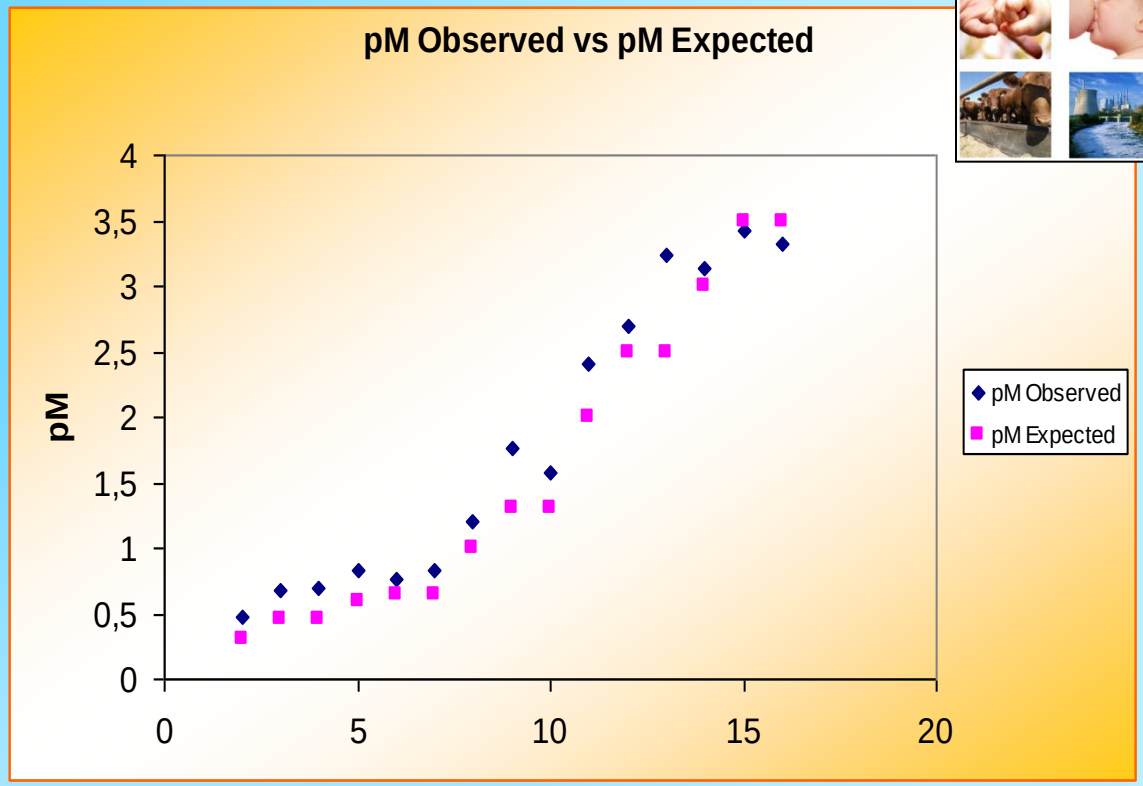
Data obtained from DR Calux and HRGC/HRMS. False positive calculation with different cut-off

STATISTICAL EVALUATION RESULTS

The statistical evaluation of the data was performed only for egg samples. It showed that the distribution was not normal; moreover the variance was not homogeneous.

So it was not possible to apply tests to establish if the difference between the two set of data was statistically significant.

pM Expected	Ratio TCDD/PCB 126	pM Observed
0,3	1:1	0,47
0,45	1:2	0,67
0,45	2:1	0,70
0,6	1:1	0,83
0,65	1:3	0,77
0,65	3:1	0,83
1	1:1	1,20
1,3	1:3	1,77
1,3	3:1	1,57
2	1:1	2,40
2,5	1:4	2,70
2,5	4:1	3,23
3	1:1	3,13
3,5	6:1	3,43
3,5	1:6	3,33



Because it was not possible to determine whether the differences found between the screening and confirmation were statistically significant, on the data obtained under controlled conditions we applied a t-test on the differences between the expected and observed values to assess if the overestimation was statistically significant. This experiment was conducted preparing solutions with different ratios of 2,3,7,8 TCDD and PCB 126.

✓There are evidences of a correlation between industrialized – urbanized areas and dioxins/PCBs contamination in raw milk

✓The experimental data obtained by simulating the contamination of the samples (different contribution between dioxins and PCBs) shows a statistically significant overestimation. Because we did not investigate all possible ratios between the concentrations of dioxins and PCBs, further studies will be needed to reach more accurate conclusions of this overestimation

✓The statistical analysis of the field samples did not allow to establish that the differences between the two methods were really sgnificant; it will be necessary to analyze more samples and to perform more detailed investigations of the contamination profiles.