



APPLICATION OF DR-CALUX TO MILK AND EGG SAMPLES: COMPARISON BETWEEN HRGC-HRMS AND SCREENING DATA

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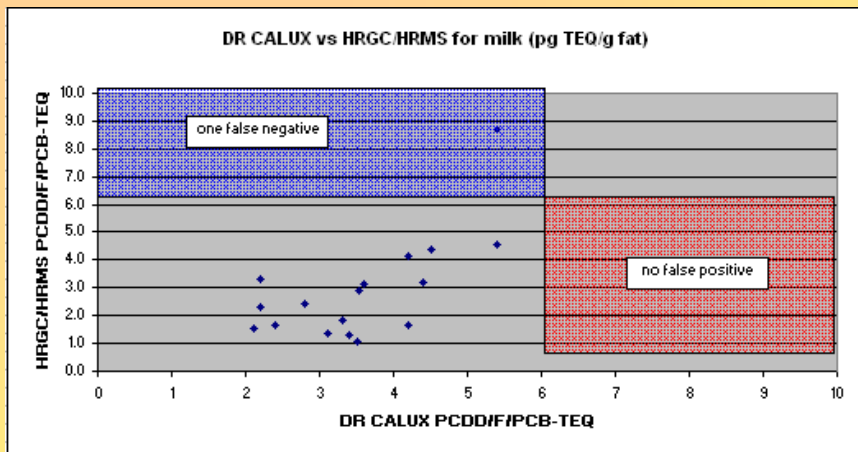
INTRODUCTION

There are clear indications that the major source of human background exposure to total PCDD, PCDF, and dl-PCB is food (more than 90 %) with food of animal origin being the predominant source. We decided to study the raw milk and eggs because they are consumed by both adult and children. In these last years consumers developed new ideas regarding food products, favouring “natural” and not-treated food, and besides, in our region is increased the consumption of the raw milk so as the request of eggs from free range hens. In order to carry out checks on a large number of samples, with low costs, our Institute 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.

We revealed an overestimation by screening related to confirmation, so the aim of this work is to compare obtained data 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.

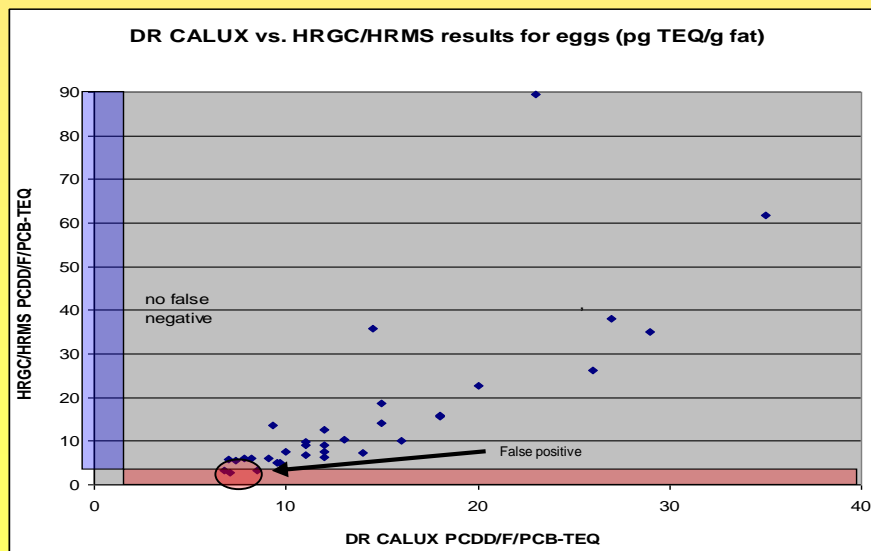


ANALYTICAL RESULTS



MILK SAMPLES		
EU limit (pg TEQ/fat)	DR CALUX (pg TEQ/g fat)	HRGC/HRMS (pg TEQ/g fat)
6	5,4	4,5
6	5,4 (false negative)	8,7
6	4,4	3,2
6	4,2	1,7
6	4,5	4,4
6	4,2	4,1
6	3,5	1,1
6	3,6	3,2
6	3,3	1,8
6	3,1	1,4
6	3,4	1,3
6	2,8	2,4
6	2,2	2,3
6	2,1	1,5
6	2,4	1,7
6	2,2	3,3

Table 1: data obtained from DR Calux and HRGC/HRMS



EGG SAMPLES					
EU limit (pg TEQ/fat)	DR CALUX (pg TEQ/g fat)	HRGC/HRMS (pg TEQ/g fat)	Cut-Off DR CALUX = Total-TEO minus 50%	Cut-Off DR CALUX = Total-TEO minus 25%	Cut-Off DR CALUX = Total-TEO 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	7,8	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	7,8	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

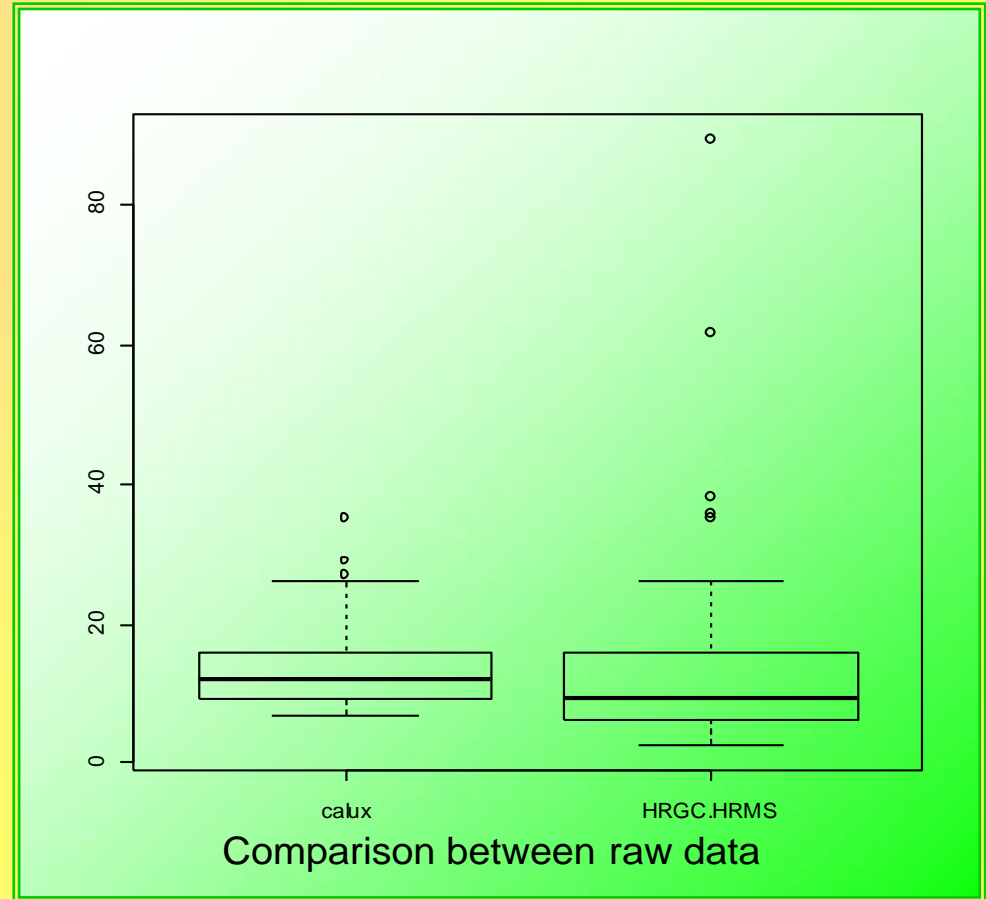
Table 2: data obtained from DR Calux and HRGC/HRMS. False positive calculation with different cut-off



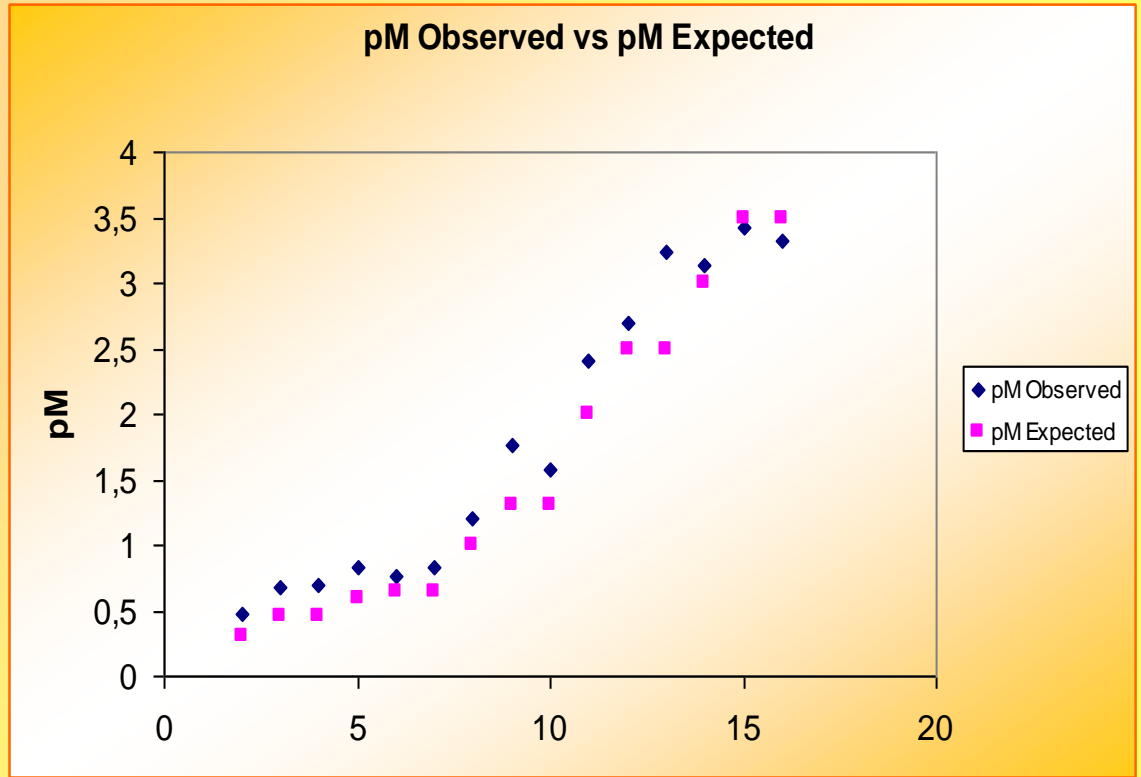
STATISTICAL EVALUATION RESULTS

The statistical evaluation of the data was performed only for egg samples. It shows that the distribution isn't normal; moreover the variance is not homogeneous.

So it isn't possible to apply tests to establish if the difference between two set of data is 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 is statistically significant. This experiment was conducted preparing solutions with different ratios of 2,3,7,8 TCDD and PCB 126 (see table). The results obtained show that the difference are statistically significant (CI = 95% 0.1056833 0.3383167)



CONCLUSION

- ✓ False negative samples were below the level established by law for screening method and the percentage of false positive was very low.
- ✓ The experimental data obtained by simulating the contamination of the samples (different contribution between dioxins and PCBs) shows a statistically significant overestimation. Because we didn't 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 didn't allow to establish that the differences between the two methods were really significant; it will be necessary to analyze more samples and to perform more detailed investigations of the contamination profiles.