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

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Introduction

Polychlorinated dibenzo-p-dioxins, dibenzofurans (PCDD/Fs) and biphenyls (dl-PCBs) are ubiquitos environmental contaminants; because their high stability they are not decomposed and show a strong tendency to biomagnification in animal and human tissues, accumulating in the whole food chain. There are clear indications that the major source of human background exposure is food (more than 90 %) with food of animal origin being the predominant source. So their monitoring is very important to prevent risks to public health, even in light of the fact that, with the globalization of markets, may be exposed to these contaminants also people living far from sources of contamination. Even if the main contributions to total PCDD, PCDF, and dl-PCB intake are due to fish and fish products (44%) and to milk and dairy products (27%) and only a 4% is due to eggs, we decided to study the raw milk and eggs because they are consumed by both adult and children. In Italy there are about 800 raw milk distributors and most of them are concentrated in Lombardia region. 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 aim of this work is to compare data obtained from screening and confirmation method. This comparison is important for two reasons; the first is to verify that the percentage of false negative is below 1%, as requested by legislation, the second is to evaluate the percentage of false positive because if they are too much, it can be heavily affected the overall cost-effectiveness of the test.

Materials and methods

For this work we analysed milk sample derived from a monitoring plane and eggs, from free range hens, these last ones only from suspected contaminated areas. A total of 600 milk samples and 50 eggs samples were analyzed with screening method. In order to apply the disposition of Regulation 1883/2006 (approximately 2 to 10% of compliant sample shall be confirmed by HRGC/HRMS) we tested 16 negative milk samples. Regarding eggs samples, 33 of the 50 analyzed presented values above the permitted limit and all of these have been sent to confirm.

DR CALUX ® screening analysis

The screening procedure was applied under an accredited QA/QC scheme. Each sample was extracted with a mixture of n-hexane/ethyl ether (97/3) (extraction is repeated for three times). The extracts were cleaned up on a double-layer silica column acidified with sulphuric acid (bottom layer: 33% sulphuric acid and upper layer:20% sulphuric acid). PCDDs and PCDFs were not separated from dl-PCBs. The final extracts were dissolved in 25 µl of DMSO. Determination of Dioxin was performed by Cell line H4IIE (from BioDetection Systems - Amsterdam), after 24 hours of incubation on a 96 wells plate; also blanks and standard (2,3,7,8 - TCDD for calibration curve) are introduced in the plate. Each extract was tested in triplicate

HRGC-HRMS confirmation analysis

The confirmation method was EPA 1613 rev.B validated in house. After lyophilisation the samples were extracted by Accelerated Solvent Extractor with toluene. The organic extract was cleaned up with an Extrelut acidified column followed by a second step with a Dioxin Prep in which we have three columns: silica gel,

alumina and graphitic carbon. Analytes were quantified on Autospec (Waters) connected to an Agilent 6890 Series gas chromatograph. Each sample was analysed in duplicate.

Results and discussion:

MILK SAMPLES

To compare the results, all analyzed samples were tested for confirmation, even if all of them showed Total TEQ values (from CALUX test) below the current EU maximum level of 6 pg TEQ/g fat, they were tested for confirmation to compare the results. Comparison data from DR Calux and HRGC/HRMS are plotted in fig. 1. Most of data, obtained from both methods, are below EU limit. The results of comparison showed that in 81 % of the samples (13) the values obtained from DR CALUX were higher than those from HRGC/HRMS while in 19 % of samples (3) they were lower. In this second group, only for one sample the value from screening has not been confirmed and we obtained data above the maximum level, as showed in fig.1 and table 1. Because our validate procedure provides that the samples must be sent to confirm when the detected level exceeds two third of the maximum permitted level, we didn't have (except one, fig. 1) false negatives. Despite having confirmed the negativity of the samples, the differences between the screening and confirmation results are significative, and it is necessary to study the profiles of contamination of the sample to understand the causes of these differences.

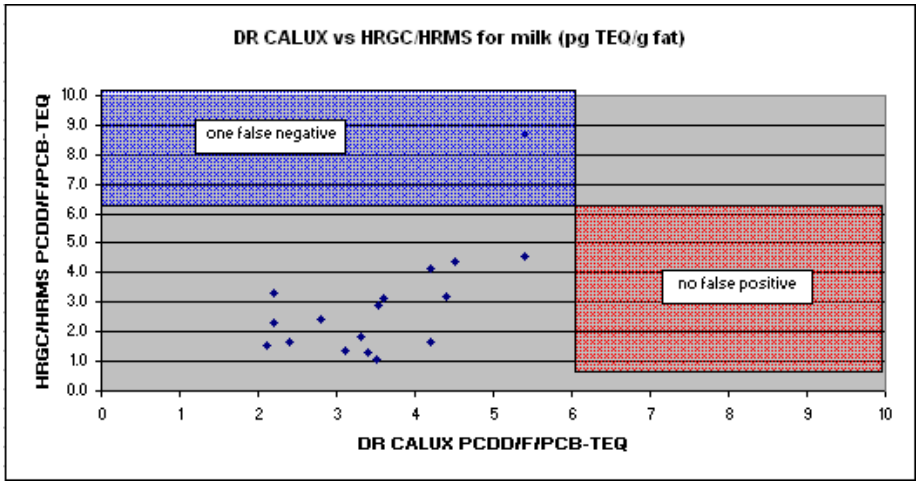


Fig. 1

	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

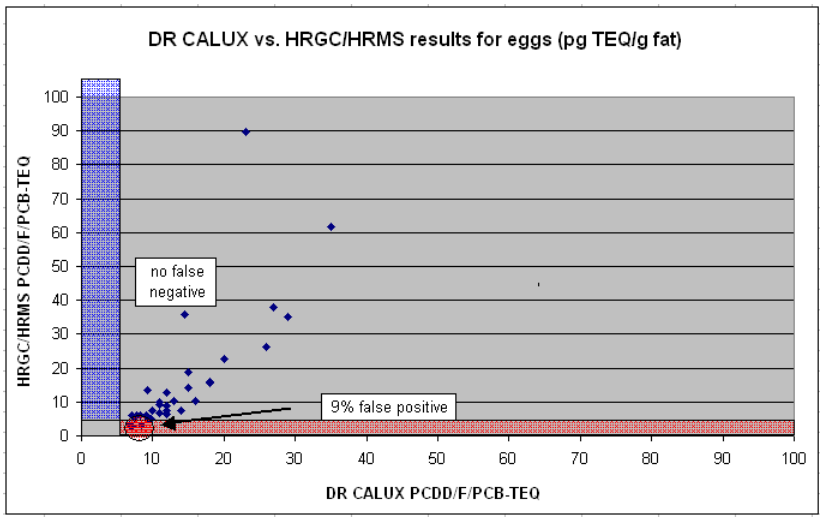
Table 1: data obtained from DR Calux and HRGC/HRMS

EGG SAMPLES

All the samples sent to confirm exceeded the 6 pg TEQ/g value with DR CALUX screening. Comparison of results between screening and confirmation showed that in 27% (9 samples), the DR CALUX has underestimated, while in 73% of the samples (24), it has overestimated the total TEQ, although the positivity is confirmed.

From a preliminary evaluation of the contamination profile, it should seem that the major differences between screening and confirmation results, are present in those sample where the contribution to total TEQ is due overall to dl-PCBs rather than to PCDDs and PCDFs.

Fig. 2



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

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

In table 2 are shown the false positive samples, obtained using different values of cut-off level, EU limit minus 50%, minus 25% and minus 2/3 (this last is the cut-off level selected in our laboratory). In the case of eggs, false positive samples are more than in the case of the milk samples and they are the same with the two last chosen cut-off levels.

The differences between screening and confirmation results are more evident in egg samples rather than in milk. These preliminary results need to be confirmed by analyzing a larger number of samples, both milk and eggs, in addition to a more detailed investigation of the contamination profiles revealed by mass spectrometry.
The results of these studies could help in defining the most appropriate cut-off, to select the samples that need confirmation before giving an opinion with respect to the limits set by the rules.

References:

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