DETERMINATION OF DIOXINS AND DIOXIN-LIKE PCBs IN FOODSTUFFS COLLECTED IN THE REGION OF VALENCIA AND PROBABILISTIC RISK ASSESSMENT OF THE DIETARY EXPOSURE.

E. Millán, O. Pardo, V. Yusà
Public Health Research Center (CSISP) – Public Health Laboratory of Valencia
Avda. Cataluña, 21. 46020 – Valencia (Spain)

INTRODUCTION

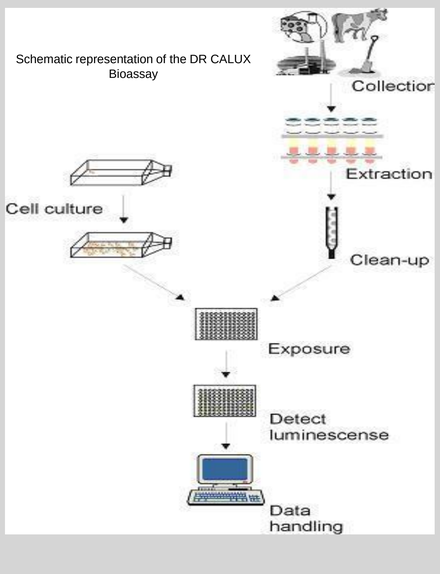
Food consumption is the main route for human exposure to polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDFs) polychlorinated biphenyls (PCBs), following accumulation of these compounds in the aquatic and terrestrial food chains. Dietary intake is estimated that accounts for at least 90% of the total exposure, with fishes and other products of animal origin making the greatest contribution. Although some authors have showed a decline of the levels in foods [1], a permanent risk assessment of the diet exposure to these highly toxic compounds in human dietary is still necessary.

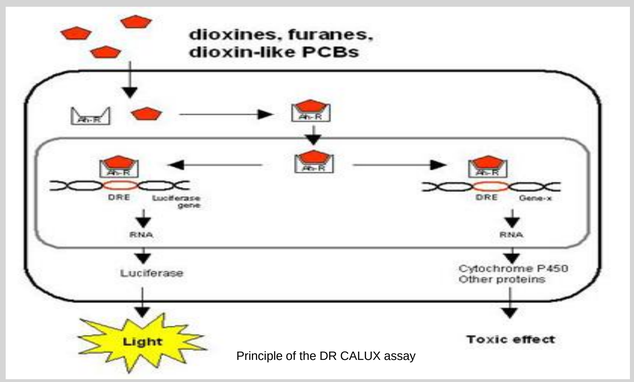
We present here the results of the levels of dioxins in the main food groups determined by the CALUX bioassay, and a probabilistic risk assessment of the exposure of the general population of the Region of Valencia.

Regulation (UE) Nº 1259/2011 of the European Comission have set maximum levels for dioxins in food and feedstuffs. To implement the regulation, Member States must undertake dioxin-monitoring programmes of food and feedstuffs, wich will require the analysis of large numbers of samples.

METHODS

DR CALUX BIOASSAY (Dioxin-Responsive Chemically Activated Luciferase Expression) is a method based on the reactivity of dioxins and dioxin-like compounds with the Aryl hidrocarbon receptor (Ahr) and afterwards of that substrate-receptor complex with the dioxin response element (DRE) in the cell nucleus.





Principle of the DR CALUX assay

SAMPLE COLLECTION
A total of 1270 composite samples were analysed corresponding to 189 individual food items that cover 90% of the adult and child diet.

CONSUMPTION DATA
Consumption data were collected from a dietary survey using a 24-h recall and performed on 1478 subjects ranging from 6-70 years old.

ANALYSIS
The CALUX approach has been used to determine the dietary exposure to Dioxins in different studies [2].

Lipid extraction is needed before the samples can be analysed with DR CALUX bioassay. The lipid extract is further cleaned over acid-silica columns. The cleaned extract containing the dioxin-like compounds, after evaporation is placed in DMSO and the cells are exposed with this extract. Following a 24 hour incubation period, cells are lysed. A luciferine containing solution (Glow Mix) is added and the luminescence is measured using a luminometer (Berthold Centro XS3).

MODELLING
For stochastic modelling of dietary exposure a Monte Carlo computational system (Creme food®) was used [4].

VALIDATION AND QUALITY CONTROL

This method have to comply with the specific characteristics considered in Regulation (UE) Nº 252/2012 of the European Comission of 21 march 2012.

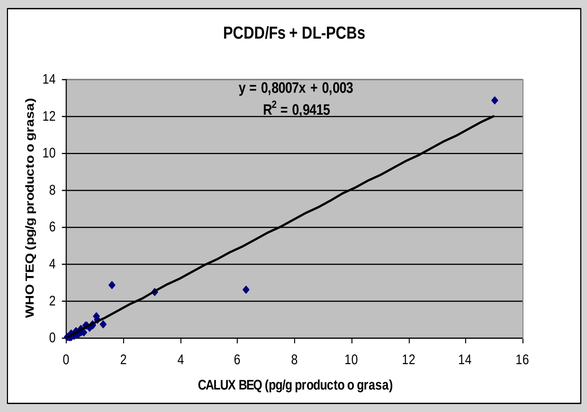
- **INTERNAL QUALITY CONTROL**

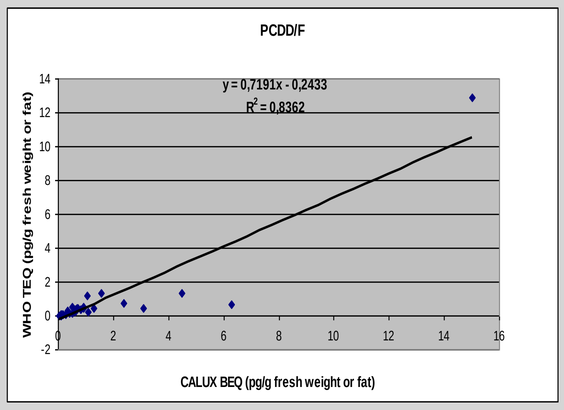
REQUIREMENTS	INTERNAL VALIDATION
PROCEDURE BLANK (<1PM)	0,6 pM
RECOVERY CERTIFIED REFERENCE MATERIAL / FORTIFIED SAMPLE.	91,45 % / 111,4 %
APPARENT BIOASSAY RECOVERY (30-130%)	83,16 %
SUPRESSION SIGNAL TEST (>75%)	118,81 %
REPRODUCIBILITY RSDR (<25%)	12,6 %
REPEATABILITY RSDr (<20%)	10,33 %
FALSE COMPLIANT RATE (<5%)	0 %

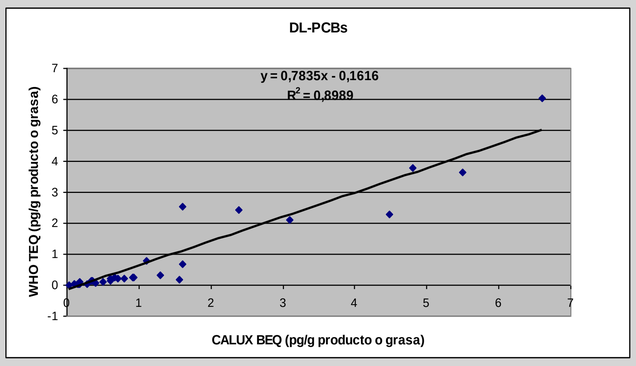
- **EXTERNAL QUALITY CONTROL**
  - **Proficiency Test EU-RL Hamburg (Germany).**
  - **BDS Amsterdam (The Netherlands)**

Interlaboratory exercises are performed since 2009. So far we have participated in 6 Proficiency tests and 1 BDS interlaboratory DR CALUX with different matrices: Animal fats, fish and fish oil, fat and vegetable fat feed, chicken eggs, pork sausage and lard, and pig meat. The result has been SATISFACTORY in all of them.

CALUX RESULTS vs. HRGC/MS RESULTS







Results obtained from the analysis of 39 food and foodstuff samples by DR CALUX and confirmed by HRGC/HRMS.
The average ratio BEQ/TEQ was 1,38.

RESULTS AND DISSCUSION

DIOXIN LEVELS

Dioxin levels found in the eleven food groups investigated in the Region of Valencia are shown in Table 1.

Table 1. Dioxins levels in the eleven foodgroups investigated (pg WHO-TEQ/g wet weight

Foodgroup	Mean	Minimum	Maximum
Alcoholic beverages	0.030	0.010	0.056
Cereals legums tubers and dried fruits	0.187	0.048	2.670
Composite food	0.230	0.014	0.810
Eggs and egg products	0.082	0.041	0.136
Fats and oils	0.367	0.132	0.627
Fish and seafood	0.686	0.124	2.590
Fruits and vegetables	0.023	0.001	0.845
Meat and meat products	0.033	0.003	0.176
Milk and dairy products	0.307	0.014	1.750
Non alcoholic beverages	0.016	0.001	0.151
Sweeteners and condiments	0.229	0.100	0.900

The food groups presenting higher contamination, expressed as toxic equivalents, were fish and seafood, fats and oils and milk and dairy products.

EXPOSURE AND RISK ASSESSMENT

For adults, the average daily intake was estimated as 1.38 and 1.56 pg WHO-TEQ/kg b.w ·day for the lower bound (LB) and upper bound (UB) scenarios, respectively.
For children, the average intake was estimated as 2.43 and 2.73 pg WHO-TEQ/kg b.w·day for the LB and UP scenarios, respectively.
The estimated intakes show that 14 % (LB) or 17 % (UB) of the children population and 4 % (LB) or 5 % (UB) of the adult population exceed the tolerable daily intake (TDI) recommended by the WHO.
The probabilistic analysis for exposure assessment permits to consider the whole distribution of exposure taking into consideration the variability in food consumption and in occurrence of pollutants in food commodities [3].
Figure 1 shows the contribution of the different foodgroups to total intake

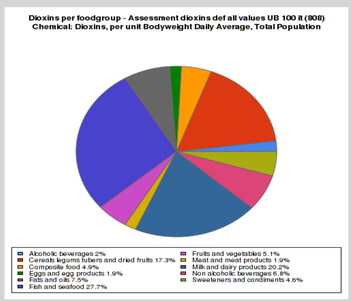


Figure 2. Contribution (%) of the different foodgroups to the total intake.

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