COMPARISON OF THE RAT AND MOUSE CELL LINES COMMERCIALLY AVAILABLE FOR CALUX BIOASSAYS

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Introduction

CALUX (Chemical-activated luciferase gene expression) is nowadays more and more widely used, both for the control of the norms applied to the food chain and to environmental contamination evaluation. In that purpose, two cell lines are commercially available: one rat cell line, commercialized by Bio Detection System (BDS, The Netherlands) and one mouse cell line, commercialized by Xenobiotic Detection System (XDS, USA). Both suppliers propose different clean-up methods and a slightly different method in the preparation, dosage and reading of the plates.
Until now, almost no comparison of the cell lines has been performed, or the comparison includes many variables (extraction, purification, method of preparation, dosage and reading of the plate) so that it is difficult to evaluate which variables are mainly responsible of the observed differences.
The objective of the research presented here is to perform a direct comparison of the 2 cell lines, and evaluate which variables can be responsible of the discrepancy observed between the results.

Materials and methods

The same sample of cod liver oil was purified by the CART and by the ISP on an acidic silica column (clean-up procedure usually applied when using the rat cell line). The sample was also purified on an a acidic silica column + a carbon column by the ISP; this purification leads to the separation of the extract in a dioxin fraction containing all PCDD/F and a PCB fraction containing all coplanar PCB (cPCB) and about 30% of the mono-orthos PCB (clean-up procedure usually applied when using the mouse cell line) (Figure 1). Sufficient amount of fat was purified so that all experiments can be performed with the same extract.

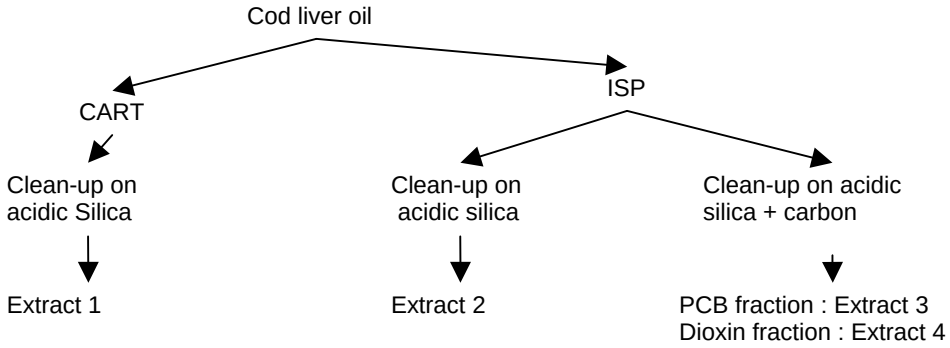

Figure 1: Sample preparations applied for the cod liver oil sample

Each extract was then analyzed in triplicate (on 3 different plates) using the mouse or the rat cell line. As the methods of preparing, dosing and reading the plate are slightly different from one lab to the other, each lab applied his own method (CART or ISP method), using the rat or mouse cells (Figure 2.).

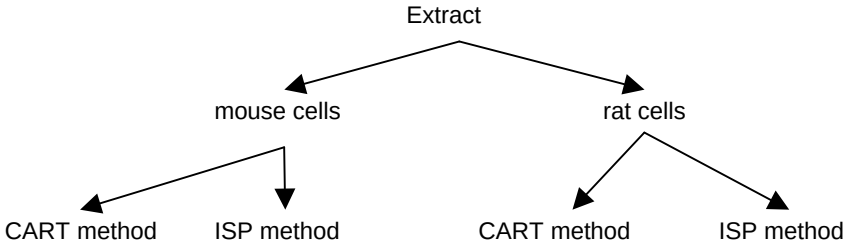

Figure 2: Different ways to analyze the same extract

Twelve samples of mussels were then analyzed, each lab applying the procedure proposed by the supplier of the cells. A detailed description of the procedure applied for CALUX analysis is given elsewhere [1,2]. A detailed description of GC-HRMS analyses can be found in Focant et al. [3]

Results and discussion

1. Sensibility

Using the same calibration solutions and the same luminometer, the ratio of the rat EC50 to the mouse EC50 is 4.6, indicating that the rat cells are about 5 times more sensitive than the mouse cells.

2. Comparison of methods

Results obtained for the same extract, analyzed by the same cells but using the different methods of preparation, dosage and reading of the plate are very closed (Table1), except for the extract 2

analyzed by the rat cells, and the PCB fraction. The difference may be related to some losses by evaporation of the PCB (the concentration under N_2 used by the CART method is softer than the concentration in a centrifuge under vacuum used by the ISP method).

		Concentration (pg TEQ/g fat)	
Extract	CALUX cells	ISP method (%RSD)	CARTmethod (%RSD)
Extract 1 (total)	rat cells	17,2 (7)	16,5 (31)
Extract 2 (total)	rat cells	38,0 (3)	27,1 (26)
Extract 3 dioxin	rat cells	18,1 (11)	18,3 (21)
Extract 4 PCB	rat cells	8,3 (6)	17,9 (23)
Extract 1 (total)	mouse cells	9,6 (26)	10,8 (20)
Extract 2 (total)	mouse cells	12,9 (6)	14,2 (37)
Extract 3 dioxin	mouse cells	19,4 (9)	15,4 (5)
Extract 4 PCB	mouse cells	6,5 (17)	10,7 (27)

Table 1. Comparison of results obtained for different extracts, cells and methods. Each extract was analyzed on 3 different plates (2 wells per plate).

The RSD associated with the triplicate measurements (same extract analyzed on 3 different plates (2 wells per plate)) are however lower with the ISP method than with the CART method. This may be due to the fact that the ISP has more experience in CALUX analysis than the CART.

3. Comparison of the cells

In order to ensure that only the cells responses are compared, results presented in figure 3 are obtained for the same extract, using the same method (ISP method since the RSD are lower), with the same calibration solutions; only the cells are different.

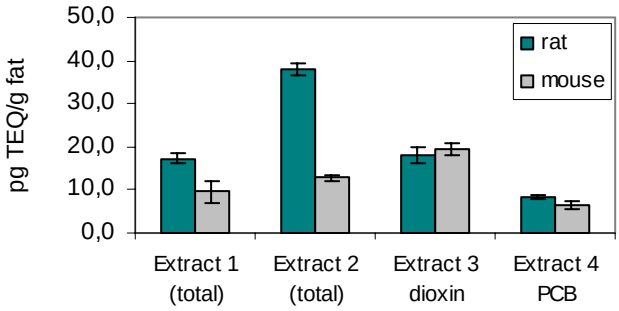

Figure 3. Comparison of the CALUX results, for the same extracts, using the rat or mouse cell lines

Different observations can be made:

- Results obtained by the 2 cell lines for the extract 3 (dioxin fraction) are very close. The RSD for the 12 (4 triplicate) measurements of this fraction (2 cell lines and 2 methods) is only 10%.
- Results obtained for the PCB fraction are more variable (Table 1). As stated previously, the difference between methods may be related to some losses during the concentration step. The difference between cell lines (cart method, Table 1) may also be related to the difference in REP, especially for the REP of PCB126 : REP= 0,038 for the mouse cells [4] and REP=0,065 for the rat cells according to Behnisch & al.[5], or 0,04 according to Scippo & al. [6].
- For the extracts 1 and 2, collected just after the acidic silica column, results obtained with the mouse cells are always lower than with the rat cells. We think that the main reason is the toxicity of these extracts for the mouse cells. To illustrate that, the extract of a procedural blank (purification on an acidic silica column) was concentrated in 4µl of a TCDD solution in DMSO. The response of the procedural blank + standard solution was measured as well as the standard solution alone. The 2 responses should be the same, but the response of procedural blank + standard solution is always significantly lower than the standard solution alone. Consequently, this procedure is not accepted and never used at the ISP with the mouse cells.
- If only the results obtained with the rat cells are considered for the extracts 1 and 2, results vary between 16,5 to 38 pg TEQ/g. Again, the variation observed may be related to some toxicity of the extract to the cells. Indeed, the responses of the rat cells are different when different amounts of the extract 1 are used for the measurements (respectively 25,3 and 17,2 pg TEQ/g fat), but are the same when different amounts of the extract 2 are used for the measurements (38 pg TEQ/g fat). As the purification of the extract 1 and 2 were the same, but were performed in different laboratories, with different adsorbents and solvents, the toxicity may be introduced by the solvents and/or adsorbent, as described before.

4. Comparison of the clean-up procedure.

The toxicity introduced by the procedure (solvents and adsorbents) seems to play an important role in the difference observed between the 2 cell lines. A clean-up using only a silica column leads to extracts that cannot be analyzed by the mouse cells, at least according to the quality control applied at the ISP. It seems that the rat cells are also sensitive to this toxicity, even if it is to a lesser extent. When a carbon column is placed in serie with the acidic silica column, the extracts collected (PCB and dioxin fractions) are cleaner and quite similar results are obtained for the 2 cell lines. It seems then very important to apply quality control in order to check the toxicity of the purified extract (one example of quality control is presented in ref 1).

Besides this, more compounds are discarded when a carbon column is used (the hexane flowing trough the carbon column is discarded as well as the first fraction (hexane-acetone) eluted from the carbon column). The discarded fractions contain AhR agonists (part of the mono-orthos PCB for example) as well as AhR antagonists (PCB and hexachlorobenzene for example), so that the response of the dioxin + PCB fractions can be inferior or superior to the response of an extract cleaned on an acidic silica column only.

Also, the separation between dioxin and PCB leads to more information than the total of both fractions.

5. Comparison of results obtained by the 2 CALUX for samples of mussels.

Twelve samples of mussel fat were analyzed by GC-HRMS and by CALUX. For the CALUX analysis, each lab applied the recommendation of the supplier: clean-up on acidic silica + carbon columns with the mouse cells at the ISP (ISP method) and clean-up on acidic silica with the rat cells at the CART (CART method).

The CALUX responses expected for PCDD/F and cPCB can be calculated by multiplying the concentrations measured by GC-HRMS by the CALUX REP [4,5,6]. These responses are compared to the chemical responses using the WHO- TEF for human[7] in the figure 4;

- The coplanar PCB 126, with a WHO-TEF of 0.1, is responsible of about 80% of the PCB TEQ in chemical measurements. The REP of this PCB is 0,038 for the mouse cells and CALUX measurement for the PCB fraction is then expected to be approximately one third of the chemical measurement. The same is observed for the rat cell line if the REP values published by Scippo & al. are considered. If the REP values published by Behnish are considered, CALUX measurement is expected to be approximately two third of the chemical measurement (Figure 4A).
- For the PCDD/F, the differences between the REP of the rat cells and the WHO-TEF leads to an overestimation of about 40% of the CALUX response when compared to the chemical response. The REP of the mouse cells are very close to the WHO-TEF for human and the expected CALUX response is extremely close to the chemical measurement (Figure 4B).
- When the sum of cPCB and PCDD/F is considered, the overestimation of the PCDD/F by the rat cells compensates the underestimation of the cPCB, and the sum is quite close to the chemical measurement. For the mouse cells, the sum of PCB and PCDD/F is underestimated due to the underestimation of the cPCB compared to chemical analysis. The CALUX response expected with the rat cells is 1.5 to 1.8 times higher than the CALUX response expected with the mouse cells (Figure 4C).
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The actual CALUX measurements of the mussel samples are compared to chemical measurements in figure 5.

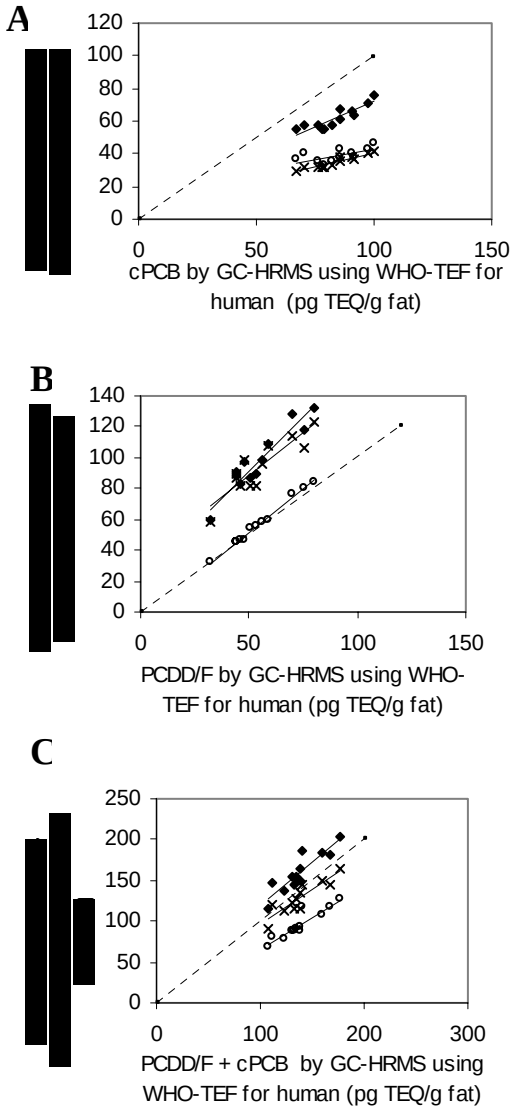

Figure 4. Comparison of expected CALUX responses (x REP for rat from ref 6; ♦ REP for rat from ref 5; O REP for mouse from ref 4) and chemical measurements for mussel samples. ----- x=y

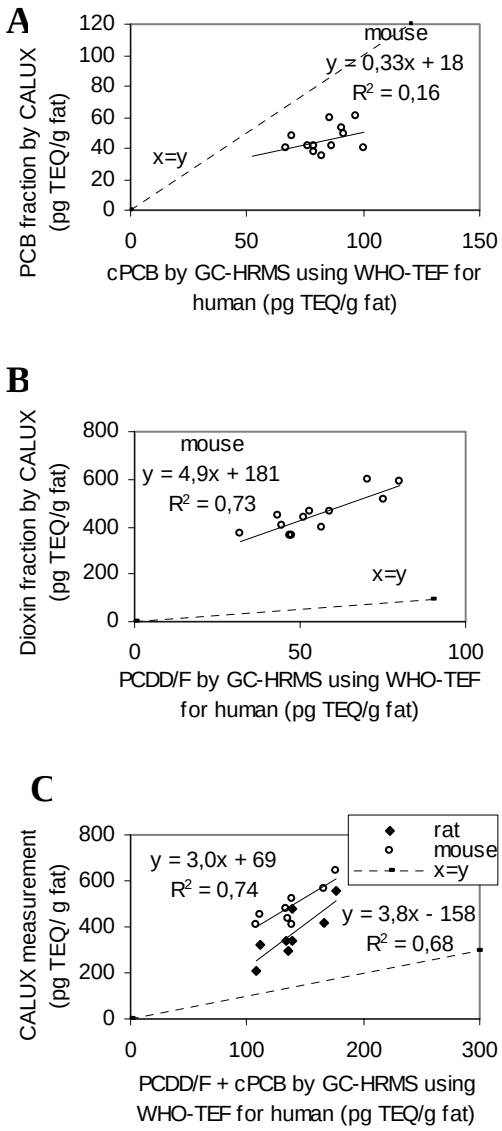

Figure 5. Comparison of CALUX with the chemical measurement for mussel sample

For the CALUX using the mouse cells, two measurements are performed:

1. the measurement of the PCB fraction (Figure 5A) represents about 30% of the chemical measurement. The difference is due to the difference between REP and WHO-TEF of the cPCB (as illustrated in Figure 4A), and probably no other AhR ligands contribute to the response.
2. the measurement of the dioxin fraction (figure 5B) leads to much higher results than those expected if only PCDD/F were present in that fraction (as illustrated in Figure 4B). Since WHO-TEF and REP are close for PCDD/F, the difference between the CALUX measurement of the dioxin fraction and the chemical measurement of the PCDD/F is due to the presence of other AhR ligands.

For the CALUX using the rat cells, only one measurement is performed on the extract collected after the acidic silica column. This measurement is always lower than the sum of the PCB and dioxin fraction measured by the CALUX using the mouse cells, even if the opposite is expected based on the difference between REP (Figure 4C and 5C). The difference may be due to some toxicity of the extract collected after the acidic silica column and/or to the presence of more antagonistic effects when all compounds are analyzed in one fraction. However, the measurements are in the same order of magnitude and the CALUX results are both correlated to the chemical results. The ecotoxicological interpretation of the results would then probably lead to the same conclusions.

Conclusions

Some differences exist between measurements performed by CALUX using the mouse cell line or the rat cell line. Before any conclusions can be proposed, the potential toxicity of the extract has to be checked carefully by the setting up of strict quality control criteria, to avoid bias of the measurements. The difference in the method of preparing, dosing and reading the plate does not seem to play an important role. The differences between REP may play a non negligible role, and the influence of this parameter on the final results would depend on the ratio between the different AhR ligands. The different clean-up proposed by the suppliers lead also to different information: the addition of a carbon column after the acidic silica column increase the analysis time, but lead to cleaner extracts and to more information on the ratio of dioxin like compounds.

References

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