

CALUX CALIBRATION IN LOW CONCENTRATION RANGE

Sanctorum H, Elskens M, Goeyens L, Baeyens W, Schroyen C

Vrije Universiteit Brussel (VUB), Laboratory of Analytical and Environmental Chemistry, Pleinlaan 2, 1050 Brussels, Belgium.

Introduction

CALUX (Chemical Activated LUciferase gene eXpression) is a reporter gene mammalian cell bioassay that has been validated for several matrices, such as milk¹, blood plasma² and marine biological matrices³. The recombinant cells used in the CALUX bioassay contain a stably transfected AhR-responsive firefly luciferase reporter gene, which responds by the induction of luciferase. The measured luminescence is converted into a bioassay toxic equivalency value (CALUX-TEQ) by the comparison of the response for a given sample to a dose-response curve obtained with 2,3,7,8-TCDD standards^{4,5}. Generally, a four parameters Hill-plot is used to fit a sigmoid curve through the standard solutions. Only the steep linear region of this curve is used to determine CALUX-TEQ values with high accuracy^{5,6}. However, samples often contain low concentrations of dioxin-like compounds and are often available in small amounts (e.g. in biomonitoring studies), resulting in responses located in the lowest curved part of the sigmoid calibration curve⁷. As a consequence, CALUX-TEQ values can be determined less accurately. To solve this problem and to define a lower detection limit, data analysis has been done by a linear calibration curve through low TCDD solutions.

Material and methods

Ten calibration solutions of 2,3,7,8-tetrachlorodibenzo-p-dioxin in dimethylsulfoxid (50,000 , 25,000 , 12,500 , 6,250 , 3,125 , 1,562 , 781 , 391 , 195 and 98 fg TCDD in 2 μ L DMSO per well) were analyzed twice on each cell plate to determine a sigmoid calibration curve. In addition, 9 calibration solutions of TCDD (781.2 , 558.0 , 390.6 , 279.0 , 195.3 , 139.5 , 97.7 , 69.8 , 48.8 , 34.9 and 24.4 fg TCDD in 2 μ l DMSO) were measured three times on each plate. These 9 solutions were used for linear calibration.

Two quality control solutions were each added to 4 wells on each plate as a measure of the variability of the method and cells (0.15 pg and 0.3 pg TCDD each in 2 μ l DMSO). A blank solution of 2 μ l DMSO was added to 4 wells on each plate. In total, 5 cell plates were analyzed.

Results and discussion

The sigmoid calibration curve (figure 1) has a working range between 0.78 and 3.13 pg TEQ per well. In this area, the dose-response curve (with the logarithm of the TCDD concentration) is considerably linear and has the highest sensitivity due to the steepness. Outside this region (down- or uphill) the sample responses are less reliable since a higher variability occurs^{2,5,6,8}.

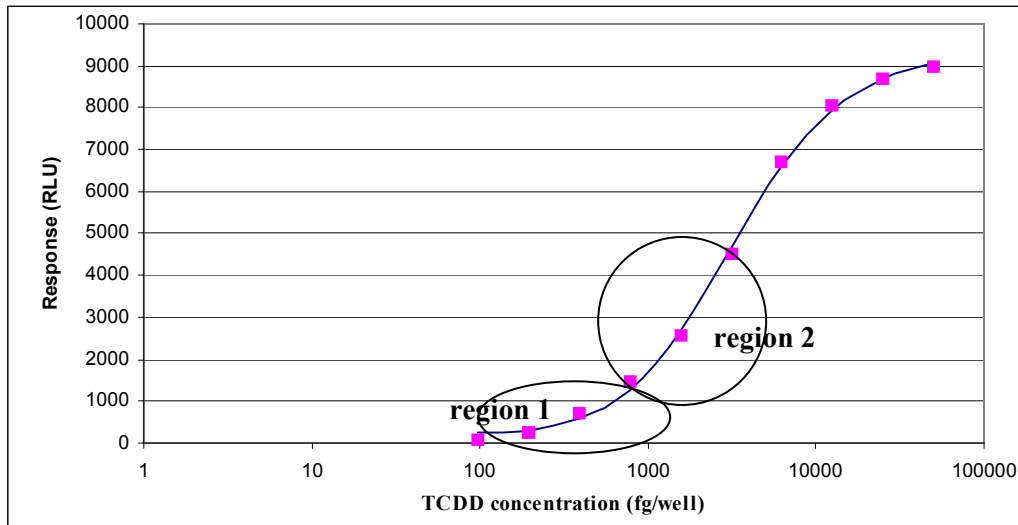


Figure 1: Sigmoid curve for generally used calibration solutions. Region 1 shows the area where it is better to replace a sigmoid fitting by a linear calibration; region 2 is the reliable part of the sigmoid calibration curve

This aspect of non-linearity is a consequence of the lack of uniformity of the coordinate system on the solution locus. Changing the parameterization will result in a new coordinate system on the solution locus that corresponds to a new set of regression parameters and displays a better estimation behavior. Within the TCDD concentration range 24.4 to 781.2 fg TCDD per well, a linear response curve could be fitted with the following equation:

$$y = 2,986 x + 480$$

The sensitivity of the model is expressed by the slope, which is 3000 ± 200 RLU/pg TCDD. The 2 quality control solutions gave 0.16 ± 0.02 pg TEQ and 0.29 ± 0.04 pg TEQ, resulting respectively in a recovery of 105% and 97%.

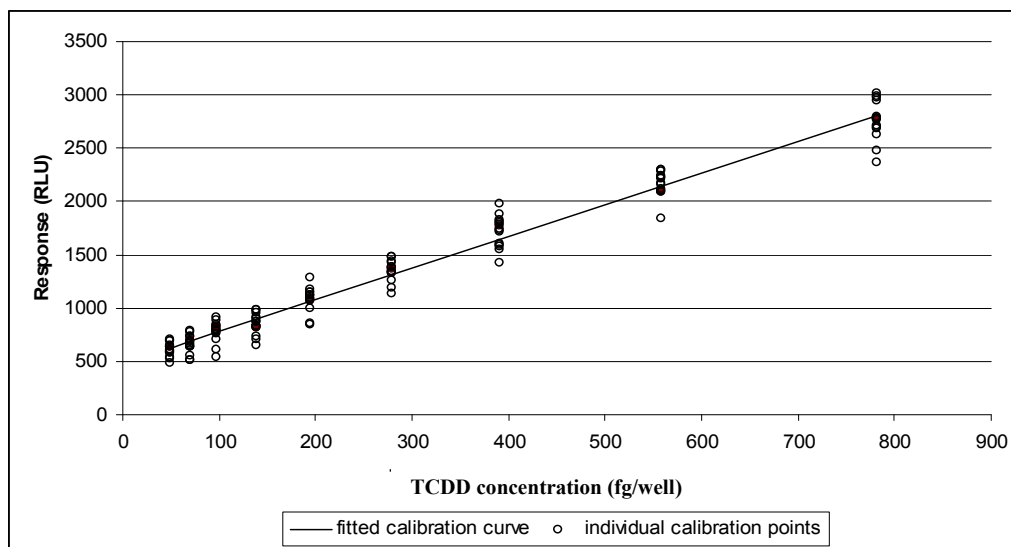


Figure 2: Linear curve for low concentrations

The detection limit was calculated as the mean response of the blank + 3 times its standard deviation⁹; this corresponds to a response of 832 RLU or 0.12 pg TEQ/well, using the equation mentioned above. The detection limit is almost three times lower than the limit of quantification determined by the sigmoid curve (0.3 pg TEQ/well⁷). The use of a linear calibration curve also allows the determination of an uncertainty on the calculated TEQ values. As a precision test, the standard deviation associated with the concentration was determined¹⁰. The relative standard deviation is given in table 1 and only exceeds 20% for concentrations lower than the detection limit.

Concentration (fg TEQ/well)	Response (RLU)	SD _{TEQ} (pg TEQ/well)	RSD (%)
781.3	2813	24.2	3.1
558.0	2146	23.5	4.2
390.6	1646	23.2	5.9
279.0	1313	23.1	8.3
195.3	1063	23.2	11.9
139.5	897	23.2	16.7
97.7	772	23.3	23.9
69.8	688	23.4	33.5
48.8	626	23.4	47.9

Table 1: Standard deviations for low calibration solutions

Conclusion

Depending on the concentration range of the samples, a model can be chosen for calculation of the CALUX-TEQ of that sample. For concentrations between 0.12 and 0.78 pg TEQ/well it is appropriate to use the linear calibration curve while for concentrations between 0.78 and 3.13 pg TEQ/well it is better to use the sigmoid curve. In case concentrations are too high, one has to dilute.

Acknowledgements

This project is funded by the Interuniversity Attraction Poles (Research Project P6/13), supported by the Belgian Science Policy.

The authors are grateful to the members of the Scientific Institute of Public Health.

References

1. Van Overmeire I., Van Loco J., Roos P., Carbonnelle S. and Goeyens L. *Talanta* 2004; 63: 1241–1247.
2. Van Wouwe N., Windal I., Vanderperren H., Eppe G., Xhrouet C., Massart A.C., Debacker N., Sasse A., Baeyens W., De Pauw E., Sartor F., Van Oyen H. and Goeyens L. *Talanta* 2004; 63: 1157–1167.
3. Windal I., Van Wouwe N., Eppe G., Xhrouet C., Debacker V., Baeyens W., De Pauw E. and Goeyens L. *Environmental Science and Technology* 2005; 39: 1741.
4. Van Overmeire I., Clark G.C., Brown D.J., Chu M.D., Cooke M., Denison M.S., Baeyens W., Srebrnik S. and Goeyens L. *Environmental Science and Policy* 2001; 4: 345–357.

5. Windal I., Denison M.S., Birnbaum L.S., Van Wouwe N., Baeyens W. and Goeyens L. *Environmental Science and Technology* 2005; 39: 7357.
6. Sanctorum H, Windal I, Hanot V, Goeyens L, Baeyens W. *Archives of Environmental Contamination and Toxicology* 2007; 52: 317–325.
7. Schroyen C, Van Wouwe N, Sanctorum H, Goeyens L, Baeyens W. *Organohalogen Compounds* 2006;68: 2511.
8. Brown D, Goeyens L, Van Overmeire I, Chu M, Murata H, Clark G. *Organohalogen Compounds* 2001; 54: 32-35.
9. Currie L.A. *Pure Applied Chemistry* 1995; 67: 1699-1723.
10. Miller J. and Miller J. *Statistics and Chemometrics for Analytical Chemistry* 2005; 107-149.