ANALYTICAL CHARACTERISTICS OF THE H1L6.1 AND THE H1L7.5 MOUSE HEPATOMA CELL LINES

Van Langenhove K.[1], Croes K.[1], Denison M. S.[2], Baston D. S.[2], Elskens M.[1], Baeyens W.[1]

[1]Vrije Universiteit Brussel (Free University of Brussels), Department of Analytical and Environmental Chemistry (ANCH), Pleinlaan 2, 1050 Brussels, Belgium.
[2]Department of Environmental Toxicology, Meyer Hall, University of California, Davis, CA 95616, USA.

Introduction

Polychlorinated dibenzo-*p*-dioxins, -furans and biphenyls form a group of ubiquitous environmental contaminants that persist in time, accumulate in a non-polar matrix as well as biomagnifie in the trophic chain[1]. Due to the potency of the most toxic congener, the 2,3,7,8-TCDD, only very small amounts are needed to exert health effects. It is therefore needed to have sensitive tools that allow quantification of these low amounts of toxic compounds in biological and environmental samples.

One of the most utilized cell lines at this point is the H1L6.1 cell line[2,3], a wild type Hepa1c1c7 cell that has been stably transfected with a luciferase gene under control of the Aryl hydrocarbon receptor, allowing induction of luciferase by dioxin and dioxin-like compounds in a time-, dose-, ligand- and AhR-dependent manner.

The more recently developed H1L7.5 cell line[4] differs from the previous one because of the number of inserted DRE's (Dioxin Responsive Elements). These increased from 4 (H1L6.1) to 20 (H1L7.5), where 4 DRE's constitute one DRD (Dioxin Response Domain). Increasing the number of DRE's under control of the AhR augments the light output for each molecule of TCDD that binds to the AhR. Hence, with these third generation (G3) luciferase bioassays, lower amounts of TCDD and other related compounds can be distinguishable from basal levels, while they were previously undetected by the commonly used H1L6.1 cell line.

This newly developed cell line could be particularly of interest in analysis of blood samples[4] and low detectable contamination-level samples such as breast milk and deposition samples. In this article, the focus has been put primarily on these two cell lines with respect to their induction capabilities, background values and rough EC_{50} values as depicted on the graphs. A descriptive comparison of these values (as established in our lab) with literature values, (when available) will allow to assess differences between different cell lines expressing the same vector (pGudLuc6.1 or -7.5) in terms of EC_{50} and fold induction[5].

Materials and methods

Both the H1L6.1 and the H1L7.5 cell lines were investigated for their performance in relationship to 2,3,7,8-tetra-chlorodibenzo-*p*-dioxin. The same cell protocol was used when growing, seeding and dosing the plates for the H1L7.5 cell line as was previously done for the H1L6.1 line.

Protocol

Both mouse hepatoma cells were grown in α-MEM (Gibco, UK) supplemented with 10% FBS (South American; Gibco, UK) to near confluence (roughly 80 to 85% coverage of the tissue culture plate). Cells were seeded into clear bottom 96-well plates (Perkin Elmer, USA) by adding 100 µl of a homogenous cell suspension at a cell density of 7.5 10^5 cell/ml using a 12-channel multipipettor (Brand).

Cells were incubated for 20-24 hours at 37°C in 5% CO_2 and 80% relative humidity. These were afterwards dosed with a 100-fold dilution (4 µl in 396 µl of media) of TCDD (Accustandard) standard solutions as mentioned in

Figures 2 and 3. Dosing of the cells was accomplished by adding 100 µl to the wells in triplicate with all 96 wells used for 10 TCDD standards, a DMSO and media blank as well as 20 remaining positions for serial dilutions of samples.

Finally, cells were incubated for another 23-24 hours, the wells were rinsed with 50 µl of 1X PBS (Gibco, UK), checked for viability and any altered morphology, followed by addition of 50 µl of 1X lysis buffer (Promega, USA).

Afterwards, wells were read using a Glomax 96-well plate luminometer (Promega, USA) with a 5.6 second incubation time for both cell lines, after injection of 50 µl of the reconstituted luciferase assay buffer. The H1L6.1 cell line had a 5 second integration time, whereas only 3 seconds were used in the case of the H1L7.5 cell line.

For both cell lines, values are reported as the percentage of maximum RLU induced by 2,3,7,8-TCDD. The protocol described above uses a molar-derived representation for the percentage induction, where both the 1 nM and 10 nM TCDD treatment solutions are present in the data set for the H1L6.1 cell line, the 1 nM is also present in the new H1L7.5 cell line treatment set.

Results and discussion

For both the 6.1 and 7.5 cell lines, a total of 9 TCDD dose curves on individual plates were analyzed over the course of 3 different days. Results are reported on a molar basis. Graphical representations of both cell lines with full dose TCDD standard-curves are presented in Figures 2 and 3. The absolute, raw values (Figure 1) for the H1L6.1 and H1L7.5 cell lines can be checked and show a tremendously increased output in number of relative light units for the latter cells. Average (triplicate measurement) maximum values are labeled in this bar chart for reasons of comparison.

Experimental backgrounds were determined for DMSO (1% v/v) and for media. On average, a 2.3-fold increase of the blank in DMSO relative to media was observed for the H1L6.1 cell line and a 1.7-fold increase in the case of the H1L7.5 cell line (Table 1).

Table 1: Average values for the H1L6.1 and H1L7.5 cell line over a total of 9 plates.

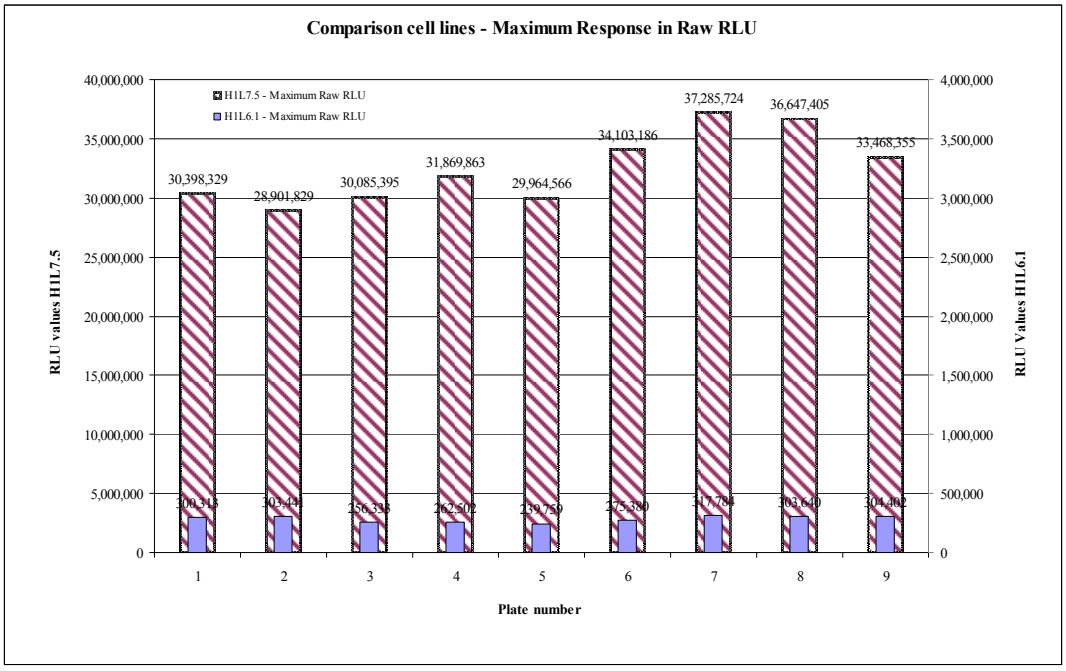
	RLU media blank	RLU DMSO blank	Maximum RLU
H1L6.1	8,000 ± 2,000	18,000 ± 3,000	280,000 ± 27,000
H1L7.5	1,750,000 ± 200,000	3,000,000 ± 200,000	32,500,000 ± 3,000,000
	Media (% Max RLU)	**DMSO Blank (% Max RLU)**	
H1L6.1	2.7 ± 0.5	6.4 ± 0.6	
H1L7.5	5.4 ± 0.4	9.5 ± 1.1	
	EC_{50} value (pM)	**LOD (% Max RLU)**	**LOQ (% Max RLU)**
	Half of maximum TCDD response	*DMSO Blank + 3,29 stdev*	*DMSO Blank + 10 stdev*
H1L6.1	~ 18-27	8.2	12.0
H1L7.5	~5.5-7.0	13.2	20.8

The blanks, either from the media or the DMSO, represent between 2.7% and 9.5% of the maximum induction of the cell lines. The fold induction, which is defined as the maximum induction divided by that of the DMSO blank, differs between the two cell lines. A low fold induction is obtained for the 6.1 line (16) and an even lower one for the G3 cell line (11). This is in contrast to values found in the literature[5] which specify factors of 22 and 24 for respectively the 6.1 and a G3 cell line[5].

Comparing the DMSO blanks for both cell lines reveals that a higher background value for the 7.5 cell line (9.5%) is present in contrary to the 6.1 (6.4%). Summation of these values (6.4% and 9.5%) and their standard deviation multiplied with an appropriate coverage factor, provide the Limit of Detection (LOD) and of Quantification (LOQ); all are mentioned in Table 1. Contradictory to what perception might suggest, the higher LOD for the 7.5 cell line compared to the 6.1 is based on a percentage maximum RLU and as such does not mean a higher LOD in terms of concentration TCDD; the 7.5 cell line is by far more sensitive.

The largest difference between both cell lines is noticeable in the raw RLU value. This is reflected in the output generated by the luminometer with values mounting to almost 40,000,000 RLUs for the 7.5 line. A comparison was made in Figure 1, where values are barely visible for the 6.1 line. These values, however, relate to the type of equipment and conditions used in our lab and by no means indicate an absolute, maximum value for this type of cell line.

Figure 1: Comparison of Raw RLU response values for the H1L6.1 and H1L7.5 cell lines.



Regarding EC_{50} values for the 6.1 cell line, they are ranging between 18 and 27 pM (Figure 2) and are in good comparison to values reported in literature: 30 pM[3]. Looking to Figure 3, a value of 5.5 to 7 pM can be extrapolated for the G3 cells.

Repeatability and reproducibility experiments were performed by having multiple plates over a period of several days. The average ± stdev (n=9) of a TCDD treatment solution of 4.95E-13 M amounted to (2.63 ± 0.54) % max RLU and (8.10 ± 1.16) % max RLU, with the former referring to the H1L6.1 cell line, the latter to the 7.5. For a treatment solution of 9.83E-10 M, these values (n=9) resulted in (97.81 ± 5.89) % max RLU for the 6.1 and (99.97 ± 4.91) % max RLU in the case of the 7.5. For both cell lines, these data points are, respectively, situated in the lower and upper plateau. Concentrations within this range showed, in general, lower absolute standard deviations and were similar to each other (data not shown). RSD values calculated (n=9) did not exceed 20%, except for the lowest standard in the H1L6.1 protocol. This does not occur with the H1L7.5 cell line since DMSO blanks and low

concentration TCDD standards yield much higher responses for a similar standard deviation, hence resulting in lower RSD values.

Future considerations include expanding the data set to have more statistical power concerning DMSO blank levels and EC_{50} values. As a next step, sample addition, originating from different matrices, can be investigated. This will allow assessment of BEQ-values for the same sample with the different cell lines.

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Figure 2: Standard curves for the H1L6.1 cell line - Percentage max. RLU vs. TCDD treatment solution.

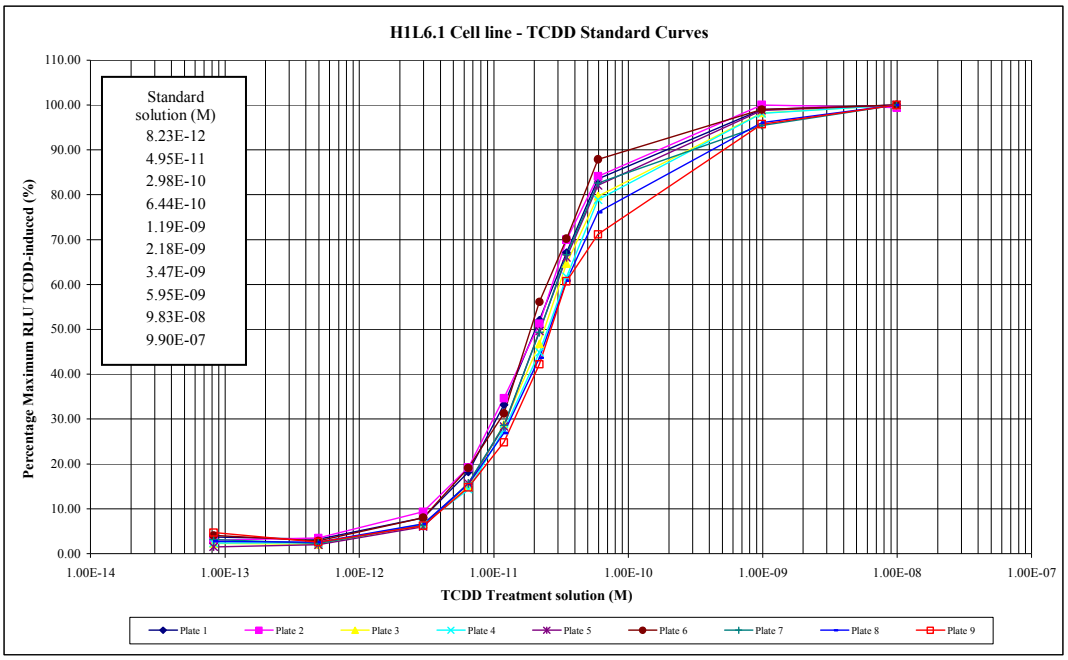

Figure 3: Standard curves for the H1L7.5 cell line - Percentage max. RLU vs. TCDD treatment solution.