NON ADDITIVE INTERACTIONS IN CALUX

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Introduction

In chemical analysis of dioxin-like compounds, concentrations of the different congeners are multiplied by their corresponding TEF. The products expressed in TEQ are summed, assuming additive contribution of the PCDD/F and PCB. However, important non-additive interactions between halogenated aromatic hydrocarbons have been described (see Safe[1] for a review). These studies mainly focused on individual polychlorinated biphenyls (PCB 52, 108, 153, 156, 159) or Aroclor 1254, which are able to inhibit the biological response induced by TCDD.

Similar non-additive interactions should be expected in CALUX analysis, since, in this methodology, a biological response is measured. However, little is known about the interactions between dioxins and other Ah ligand classes.

Several questions can be raised. Which compounds are responsible for non-additive interactions? At what concentrations? Are these concentrations usually found in the samples? Are these compounds present in the extracts or are they degraded/discarded during the clean-up? The present paper tries to answer some of these questions for compounds known to be Ah ligands or known to interact. This study focuses on hexachlorobenzene (HCB), different kinds of PCB mixtures (Aroclor) which have been used as industrial products. The Aroclor 1254, 1242 and 1260 were selected here since they are the most widely used. The industrial mixture Halowax 1014 was used for polychloronaphthalene (PCN) and the industrial mixture Aroclor 5442 was used for polychloroterphenyls (PCT).

Methods and Materials

CALUX analyses were performed using the mouse hepatoma H1L6.1 cell line developed by XDS. Solutions of the dioxin-like compounds to be investigated were prepared in hexane at different concentrations. Aliquots of these solutions were added to DMSO, and hexane was evaporated. The resulting DMSO solutions were suspended in cell culture medium and added to a monolayer of cells grown in 96 well culture plates. Before quantification, the plates were incubated for 20-24h in an incubator at 37°C, with saturated humidity and a 5% CO_2 atmosphere.
Various dioxin-like compound mixtures were first analyzed individually by CALUX, and then each dioxin-like compound mixture was analyzed in combination with a standard solution of PCDD/F in DMSO.

Results and discussion

Dose-response curves

Dose-response curves, obtained using solutions of the dioxin-like compound mixtures, were used to determine concentrations required for sufficiently high CALUX activity (figure 1). It was not possible to dissolve HCB in DMSO at every concentration tested; crystals were observed with the microscope at the highest concentrations.

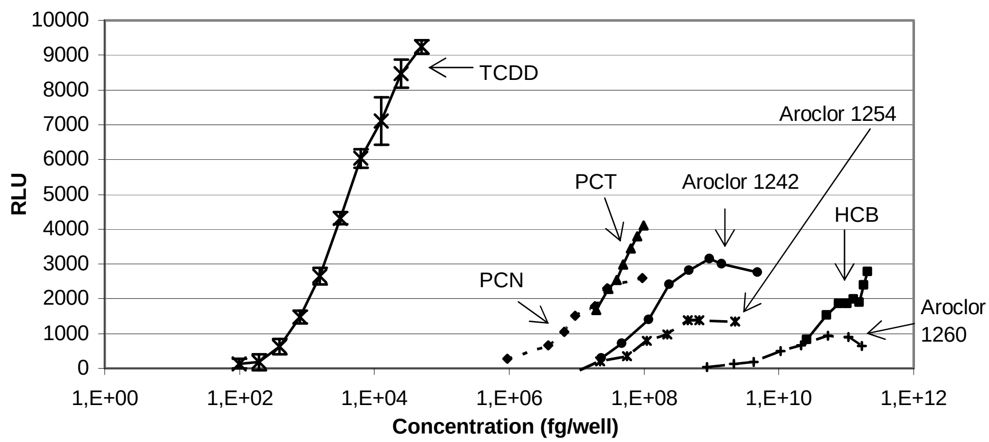

Figure 1: Dose-response curves obtained by CALUX for different dioxin-like compound mixtures.

Principle of additivity

Assuming the principle of additivity is obeyed, one should find that the response of a standard solution of PCDD/F added to the response of a dioxin-like compound solution equals the response to a mixture of the two solutions (standard solution of PCDD/F+ dioxin-like compound solution).

The validity of this hypothesis was checked for some dioxin-like compound mixtures. To simplify the experiments, a standard solution of PCDD/F of constant concentration was used. The solutions of dioxin-like compounds used to establish the dose-response curves were used in combination with the standard solution of PCDD/F. For HCB, lower concentrations were used (for which no response can be detected) to avoid the difficulty of dissolution of the HCB in DMSO.

For a better view, results are calculated and expressed as follow (figure 2 and 3): the response of the standard solution of PCDD/F and the response of dioxin-like compounds solutions were measured separately. The sum of the 2 responses is the expected response, assuming the studied compounds obey the additivity principle. The response of the mixture of both solutions is measured and expressed as the percentage of the expected response. The percentage of the expected value is then plotted versus the concentrations of the dioxin-like compounds solutions (in ng/well).

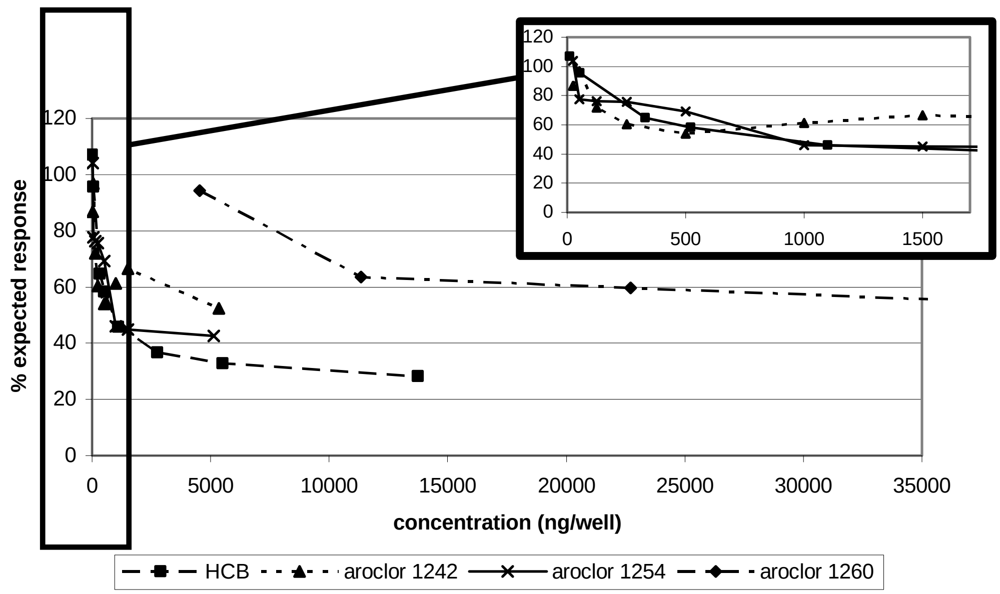

Figure 2: Deviation from additivity for HCB, Aroclor 1242, 1254 or 1260 + PCDD/F

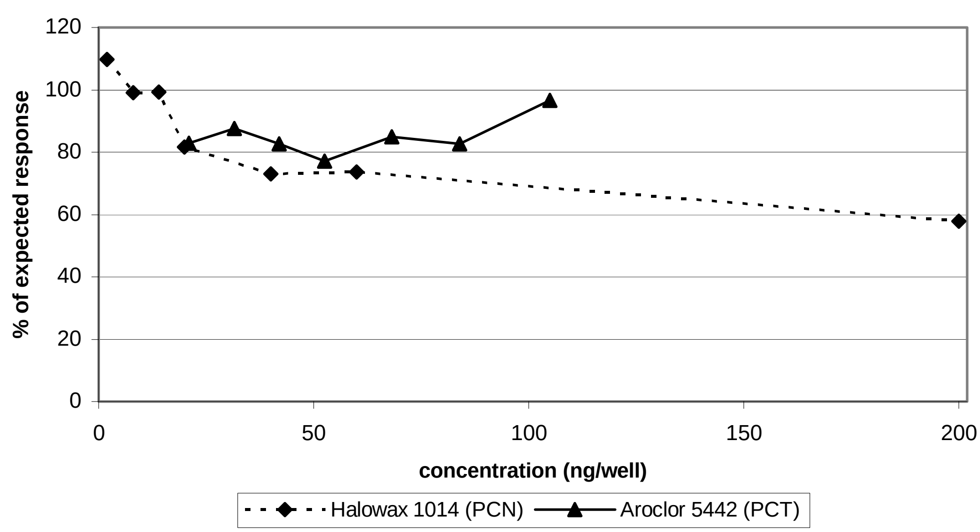

Figure 3: Deviation from additivity for Halowax 1014 or Aroclor 5442 + PCDD/F

Discussion

All compounds investigated are able to pass through the acidic silica column usually used as clean-up, and will then be present in the analyzed extract. In this study, deviation from additivity is considered as significant when the response of dioxin-like compounds + dioxins is less than 80% of the expected response (80% is used instead of 100%, to take into account the variation of the CALUX response)

The antagonistic effect of **Aroclor 1254** is well described in the literature[1,2] and has been confirmed by our experiments. **Aroclor 1242** behaves with a similar antagonistic effect in the same concentration range as **Aroclor 1254**. However, for **Aroclor 1260,** higher concentrations are required before an antagonistic effect is observed.

To avoid any problem of dissolution, the effect of **HCB** was tested at concentrations for which no response can be detected in CALUX. The measurement of HCB + PCDD/F should then equal the measurement of PCDD/F alone. However, a decrease in CALUX response is observed at concentrations exceeding 200 ng of HCB/well. Significant deviation from additivity was also observed for **PCN** (Halowax 1014) at concentrations above 20 ng/well. However, no significant deviation from additivity was observed at **PCT (Aroclor 5442)** concentrations tested.

The ratios of compounds (expressed as concentration) /dioxins (expressed as concentration in TEQ) needed to observe a deviation from additivity are quite high : >10000 for Aroclor, >50 000 for HCB and > 5000 for Halowax. They must be compared to the concentrations found in the different samples, since considerable variability is observed in both contamination levels and contaminant mixtures.

The clean-up applied for CALUX analyses has thus a very big importance since:

1) some Ah ligands are degraded or discarded during the clean-up (ex: PAH are degraded on the acidic silica column)
2) some compounds able to lower or suppress the CALUX response may be discarded during the clean-up (ex: PCB can be separated from dioxins by using a carbon column, and the antagonistic effect of PCB is suppressed), and the sum of the response of the different fractions of an extract may be higher than the response of the non-fractionated extract.

Conclusions

The presence of Aroclor 1242, 1254, 1260, Halowax 1014 (PCN) or hexachlorobenzene in an extract is able to lower the biological response of PCDD/F measured in CALUX. Ratios of dioxin-like compound/dioxin needed to observe this effect are high, however.

References

1. Safe, S. (1997) Teratogenesis, Carcinogenesis, and Mutagenesis 17, 285-304.
2. Harper, N., Connors, K., Steinberg, M., and Safe, S. (2001) Fundamental and Applied Toxicology 27, 131-139.