INTERACTIONS OF TCDD WITH AHR PARTIAL AGONISTS AND A COMPETITIVE ANTAGONIST: IMPLICATIONS FOR TEFS

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Abstract
Methods to predict the expected outcome of combination exposures are critical both to risk assessment and to an accurate judgment of synergism or antagonism. The Toxic Equivalency Factor (TEF) method used to assess the joint effects of dioxin-like chemicals is a special case of the method of concentration addition. However, the TEF model implicitly assumes that the individual agents are full agonists with parallel dose-response curves. Using kinetic models of simple receptor systems, we derived a generalization of concentration addition (GCA) to more accurately describe interactions of combinations that include partial agonists. The GCA and TEF models were assessed for their ability to predict activation of aryl hydrocarbon receptor-dependent gene expression by combinations of TCDD with the partial agonists PCB 105 and galangin and with the antagonist 3,3'-diindolylmethane. In each case, the GCA model fits experimental data better than the TEF model. The TEF method clearly overpredicts combination effects at the highest combination doses. At lower (environmentally relevant) doses, the difference between GCA and TEF predictions is dependent on the specific TEF values chosen, but should be small for many partial agonists.

Introduction
Environmentally relevant toxic exposures often consist of simultaneous exposure to multiple agents. Consequently, methods to predict the expected outcome of combination exposures are critical both to risk assessment and to an accurate judgment of whether combinations are additive, synergistic, or antagonistic. Concentration addition (CA) is commonly used to assess the presence of synergy or antagonism in combinations of similarly-acting chemicals and to predict effects of combinations of such agents. CA has the advantage of clear graphical interpretation: Curves of constant joint effect (isoboles) must be straight lines. The Toxic Equivalency Factor (TEF) model used to assess the joint effects of mixtures of dioxin-like chemicals is a special case of CA where the isoboles are both linear and parallel. The TEF model assumes that the individual agents are full agonists with parallel dose-response curves that differ only in potency. The CA model—and consequently TEFs—have an important limitation: they cannot be applied to effect levels greater than that achieved by any single compound included in the mixture.[1] Unfortunately, many ligands of the AhR are partial agonists.[2]

Starting with kinetic models of simple receptor systems, we have derived a generalization of concentration addition (GCA) that is intended to more accurately describe interactions of combinations of full and partial agonists.[3] Here we compare the GCA and TEF models using data on AhR-dependent gene expression produced by binary combinations of TCDD with partial agonists and an antagonist.

Materials and Methods
H1G1.1c3 cells were generously provided by Dr. Mike Denison (University of California, Davis), and were cultured and prepared for experiments as described previously with the exception that 20,000 H1G1 cells were added to each well of a 96-well, black-sided plate in 200 µl of selective medium.[4] The plates were incubated at 37°C for 24 hrs. The medium was removed and replaced with 100 µl of non-selective medium. Vehicle (DMSO, 0.5%), partial agonists (PCB105 10^{-9}–10^{-5} M, galangin 10^{-7}–$3x10^{-5}$ M), or antagonist (3,3'-diindolylmethane (DIM), $5x10^{-6}$–$4x10^{-5}$ M) were applied, immediately followed by dosing with vehicle or TCDD (10^{-14}–10^{-10} M). Each combination was dosed in 8 replicates per plate. Two columns were left untreated per plate. The plates were incubated at 33°C for 24hrs and then read in a fluorometric plate reader (Synergy2, Biotek Instruments). The excitation and emission wavelengths were 485nm (20nm bandwidth) and 530nm (25nm bandwidth). The fluorescence in all experimental wells was normalized by subtracting the fluorescence measured in wells with untreated cells. General toxicity was assessed following the fluorescence measurement by adding 20 µl of a 5 mg/ml solution of thiazolyl blue tertazolium bromide (MTT) in PBS per well, incubating for 4-6 hrs at 37°C and reading the absorbance at 570 nm. Concentrations showing toxicity in MTT were

omitted from the analysis. All doses of combination experiments with PCB105 and galangin were repeated at least three times; DIM data are preliminary based on one combination experiment. Isobolograms were generated with the contour() function in R (2.4.1). Surfaces corresponding to the GCA and TEF models were fit to data with the R nlm() routine.

The GCA equation for two agonists, *A* and *B*, is given by

$$E_{GCA} = \frac{\alpha_A [A]/K_A + \alpha_B [B]/K_B}{1 + [A]/K_A + [B]/K_B} \quad (1)$$

where K_A, K_B are macroscopic equilibrium constants (equivalent to EC_{50}s) and α_A, α_B are maximal effect levels of each ligand in the tissue or system under study (we also assume a Hill coefficient of one).[3] Under the GCA model, isoboles of agents with different maximal effects are not parallel, but have slopes—and thus relative potencies (REPs)—that vary with effect level *E*:

$$\text{slope} = \left(\frac{\alpha_A - E}{\alpha_B - E}\right)\left(\frac{K_B}{K_A}\right) \quad (2)$$

where *B* is assumed to be the full agonist and reference compound.[3] As shown by (2), isoboles of a GCA combination are negatively sloped at low effect levels, flatten as the combination effect increases, and are positively sloped (like isoboles of a competitive antagonist) at effects above the maximal effect of the partial agonist α_A. The largest REP occurs in the limit of small effect: $(\alpha_A/\alpha_B)(K_B/K_A)$. In the TEF model, the maximal effects are equal ($\alpha_A=\alpha_B$), and the slopes of all isoboles are equal with a TEF of K_B/K_A.

Results and Discussion

TCDD + PCB105

PCB105 is a mono-*ortho* substituted PCB with a WHO TEF value of 3×10^{-5}.[5] Isoboles of the PCB105/TCDD combination are negatively sloped at low doses, flatten as the effect level increases (Figure 1), and switch from negative to positive slope near TCDD 10^{-11} as predicted by GCA.

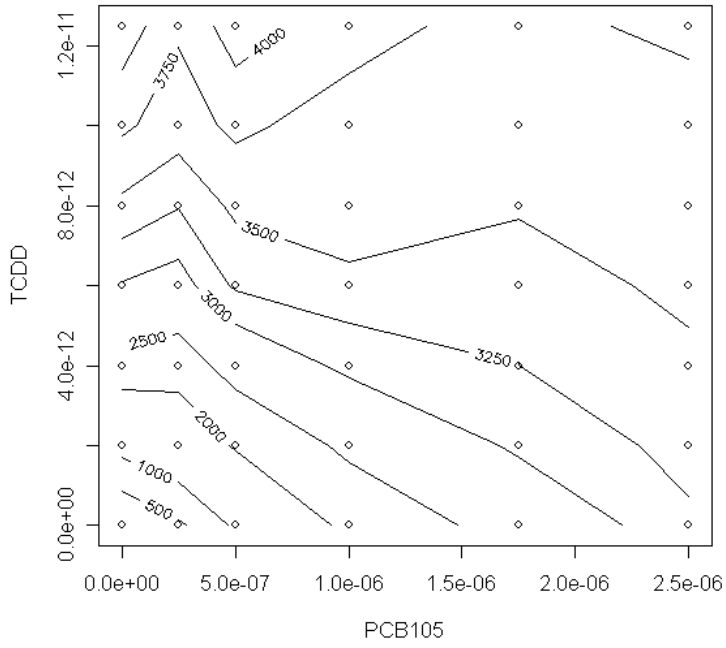

Figure 1. Isoboles of the TCDD/PCB105 combination change slope, switching from negative to positive as effect levels exceed the maximal effect of the partial agonist, as predicted by GCA. The TEF model requires parallel isoboles at all effect levels. Contours are interpolated between data points (circles).

GCA and TEF model surfaces for combination doses were constructed from the individual dose-response curves, and were compared to the experimental data; the GCA model fit better than TEF. Because of the high efficacy of this PCB105 sample (about 60% of TCDD), the dose-response curves are reasonably parallel at low doses. The relative potency estimate under a TEF model, based on the ratio of the EC_{50}s from the individual dose-response curves, was $5.4x10^{-6}$; the maximal value of the REP under a GCA model fit to the entire surface was $6.2x10^{-6}$. These values are within the range used to derive the WHO TEF.[5] The relatively high efficacy of this sample is probably due to contamination with PCB126, a common problem for commercial PCB samples.[6]

TCDD + Galangin

Although galangin does not meet the requirements for inclusion in the TEF system, its low AhR agonist activity (about 30% of TCDD in H1G1) makes it a good illustration of the GCA model.[5] Isoboles of the TCDD/galangin combination switch from negative to positive slopes near TCDD concentrations of $5x10^{-12}$, as predicted by the GCA model based on the percent activity of galangin (not shown). A GCA model surface constructed from the individual dose-response curves fit dramatically better than the TEF model surface. The TEF model fails at high doses because it cannot take into account the reduced effect that occurs when the lower-efficacy galangin competes with TCDD for receptors.

TCDD + DIM

3,3'-diindolylmethane (DIM) is a selective AhR modulator naturally found in cruciferous vegetables that has been the focus of attention as a possible chemopreventive agent against breast cancer.[7] DIM is a very weak inducer of CYP1A1. Preliminary results indicate that DIM has little or no AhR-related activity in H1G1, making it a reasonable example of a competitive antagonist of TCDD. The isoboles of the TCDD/DIM combinations are positively sloped as predicted by GCA (not shown). A GCA model surface fits the response surface well, while the TEF model surface cannot represent the competitive antagonism of DIM (not shown).

Discussion

In all three cases, the GCA model fit experimental data substantially better than the TEF model. The difference between the two models is much more pronounced for the lower-efficacy agonists. Each mixture showed a reduced effect at the highest-dose combinations; this reduction is not predicted by the TEF method, but in the GCA model can be seen as the result of competition by the partial agonist for free receptors.

The GCA model also has implications for combinations of full and partial agonists at low, more environmentally relevant doses. The model predicts that relative potencies vary with effect level and thus that a true TEF does not exist. However, the difference between the two models is not too different at very low doses: for TEFs computed from K_B/K_A (i.e., the ratio of the EC_{50}s), the effect predicted by the GCA model based on marginal data is lower than the TEF model by a factor of about α_A/α_B (the ratio of the maximal effects). For partial agonists with greater than 10% maximal effect relative to TCDD, this difference is within the order of magnitude estimate of uncertainty attributed to TEFs.[5] A number of methods have been used to estimate the REP values used to choose TEFs; the difference between the GCA and TEF models can depend on the method.[8]

In summary, the GCA method appears to accurately predict joint effects of combinations of dioxin-like agents that include partial agonists. The particular model discussed here is limited to dose-response curves that are Hill functions with a Hill parameter of 1; equivalent models can be derived for other cases. The model describes only effects moderated by receptor binding, and may not represent the complexity of a living system. The GCA approach may be useful both in classifying interactions of mixtures as well as in making predictions about their effects when the individual dose-response curves are well known. Detailed comparisons of GCA and TEF models may provide useful information for more accurately choosing TEF values for partial agonist PCBs.

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References

1. Silva E, Rajapakse N, Kortenkamp A. *Environ Sci Technol* 2002; 36(8): 1751-6.
2. Hestermann EV, Stegeman JJ, and Hahn ME. *Toxicol Appl Pharmacol* 2000; 168(2): 160-72.
3. Howard GJ and Webster TF (submitted).
4. Nagy SR, Sanborn JR, Hammock BD, Denison MS. *Toxicol Sci* 2002; 65(2): 200-10.
5. Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, Fiedler H, Hakansson H, Hanberg A, Haws L, Rose M, Safe S, Schrenk D, Tohyama C, Tritscher A, Tuomisto J, Tysklind M, Walker N, Peterson RE. *Toxicol Sci* 2006 ; 93(2): 223-41.
6. Peters, AK, Leonards, PE, Zhao, B, Bergman, A, Denison, MS, Van den Berg, M.*Toxicol Lett* 2006; 165(3): 230-41.
7. Hestermann EV, Brown M. *Mol Cell Biol* 2003; 23(21): 7920-5.
8. Haws LC, Su SH, Harris M, Devito MJ, Walker NJ, Farland WH, Finley B, Birnbaum LS. *Toxicol Sci* 2006; 89(1): 4-30.