Interactions among PCBs and TCDD as *Ah* Receptor Agonists in a Novel Human Bioassay for Cytochrome P450 IA1

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1. Introduction

There is presently a great deal of interest in the toxic effects of polyhalogenated aromatic compounds on living systems and in the development of sensitive toxicological indices for monitoring exposure to these pollutants. One of the most widely-used approaches involves monitoring alterations in xenobiotic-metabolizing enzyme systems. The cytochrome P-450 (CYP) system is foremost among such systems under study, and induction of CYP isozymes has become a widespread tool for detecting exposure to xenobiotics[1]. In particular, the CYP IA1 subfamily has been demonstrated to be highly inducible by pollutant compounds such as PAHs, PCDDs, PCDFs and PCBs. These and other compounds exert their inductive effects by binding to the intracellular *Ah* receptor which mediates *de novo* CYP enzyme synthesis. CYP induction is also used to rank compounds relative to known inducers (e.g., 2,3,7,8-TCDD) using toxic equivalency factors (TEF) calculated as an index of induction potency[2]. TEF are then used to assess the toxic hazard posed by individual compounds which occur as part of complex environmental mixtures. Furthermore, the toxicity of complex pollutant mixtures is assessed by monitoring the degree of CYP IA1 induction that the mixtures elicit. This is then compared to induction by standards (e.g., TCDD) and the toxic constituents of a complex mixture may then be quantified (e.g., as TCDD equivalents)[2]. One shortcoming of this approach to assessing toxic hazard posed by complex mixtures is that the numerous possible interactions among the constituents of a mixture are not taken into account. One major concern is that the constituents of a mixture may compete at the *Ah* receptor level resulting in a degree of CYP enzyme induction less than that which would be elicited by the compounds individually.

In order to address these concerns, our laboratory has conducted a study of interactions among *Ah* receptor agonists, namely TCDD and various PCB congeners. This study was conducted using a newly optimized bioassay for ethoxyresorufin *O*-deethylase (EROD), a catalytic marker for CYP IA1. The cell line employed in this bioassay is the human hepatoma cell line, HepG2, which has been shown to express substantial CYP IA1 enzyme activity[3]. Furthermore, in contrast to bioassays

employing non-human (e.g., murine or piscine) cell models, the HepG2 EROD bioassay is directly relevant to human health concerns. We describe below results which illustrate numerous interactions among *Ah* receptor agonists in simple (i.e., two-compound) mixtures.

Methods

HepG2 cells were obtained from the American Type Culture Collection (Rockville, MD) and were cultured and maintained at 37 °C as described previously[4]. Cells were grown to confluency on 96-well tissue culture microtiter plates and were treated with test compounds that were previously suspended in culture medium by delivery in solvent to a maximum of 1% final solvent concentration followed by vigorous mixing. Cells were exposed to test compounds for 24 hr under standard culture conditions. Subsequently, cells were washed with PBS prior to the EROD bioassay described below.

The procedure for monitoring EROD activity was a modification of the method described by Pohl and Fouts[5]. The incubation medium (final volume: 0.15 mL/well) consisted of 50 mM $NaPO_4$, pH 8.0, containing 5 mM $MgSO_3$, 10 μM dicoumarol, and 60 μM EDTA. The washed cells were pre-incubated for 5 min with 40 μM digitonin (to permeabilize cells), glucose 6-phosphate dehydrogenase (to 0.5 U/mL) and ethoxyresorufin (to 6 μM). The reaction was started by the addition of glucose 6-phosphate (to 5 mM) and NADPH (to 0.5 mM). Increase in fluorescence, representing ethoxyresorufin *O*-deethylation, was monitored in a Labsystems Fluoroskan II plate reader at a temperature of 37 °C using excitation and emission filters with wavelength optima of 538 and 591 nm, respectively. Rates of resorufin production were determined using resorufin standards. Protein content was subsequently determined using the Lowry method.

Results

HepG2 cells demonstrated induction by (2,3,7,8-)TCDD with a high degree of sensitivity and with a maximal response at 5 nM TCDD. This compound was then used as the prototype *Ah* receptor agonist in subsequent studies of interaction with various PCB congeners administered simultaneously to the cells. When HepG2 cells were exposed to TCDD in the 0-2.5 nM concentration range, a graded EROD induction response was observed (Fig 1). When PCB #77 was administered to cells without TCDD present, no significant induction was observed at concentrations up to 500 nM (Fig. 1). However, when this PCB congener was co-administered with TCDD, a marked inhibition of the TCDD-mediated EROD induction was observed. Furthermore, this inhibition was observed at all TCDD concentrations tested (0.3-2.5 nM) and was more pronounced at higher TCDD concentrations (Fig. 1). Qualitatively similar results were observed when PCB #169 was tested in combination with TCDD using the same dosage regimen (Fig.2). These results indicate that, although PCB #'s 77 and 169 are not effective EROD-inductive agents when administered alone, they are effective inhibitors of the EROD induction response mediated by *Ah* receptor agonists like TCDD.

In contrast to results described above, PCB #126 elicited significant EROD induction when administered in the absence of TCDD (Fig. 3). When this PCB congener was co-administered with TCDD at lower concentrations (i.e., 0.3-1.25 nM TCDD), a suppression of the TCDD-mediated

induction was evident (Fig. 2). Conversely, at higher TCDD concentrations (1.25 nM), the PCB-mediated induction was inhibited by TCDD. At the highest TCDD concentration (2.5 nM) tested, PCB #126 exhibited a dose-dependent suppression of the TCDD-mediated EROD induction. These results indicate that PCB #126 is both an effective *Ah* receptor agonist when administered alone and is also an effective competitor for induction in the presence other agonists such as TCDD.

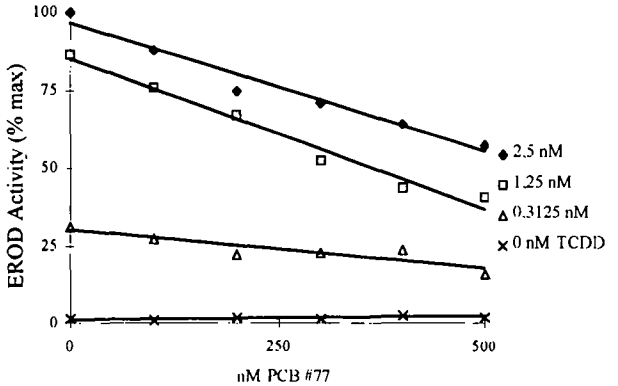

Figure 1. Interaction of PCB #77 and TCDD in the HepG2 EROD bioassay.

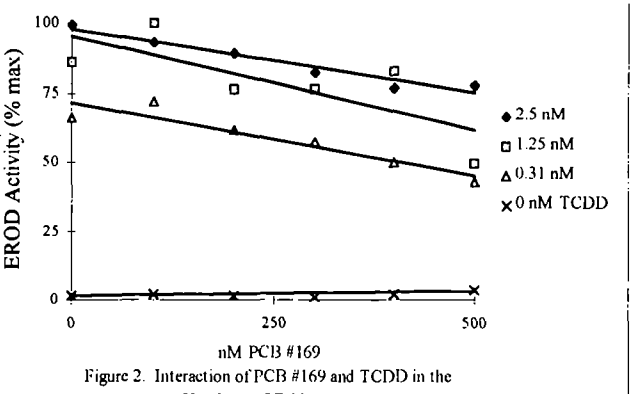

Figure 2. Interaction of PCB #169 and TCDD in the HepG2 EROD bioassay.

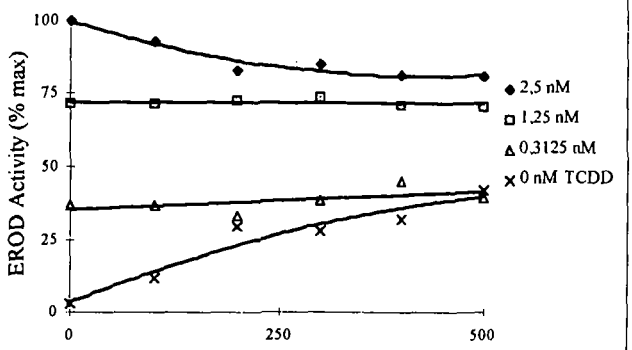

Figure 3. Interaction of PCB #126 and TCDD in the HepG2 EROD bioassay.

Discussion

The toxic hazard posed by the great variety of polycyclic and polyhalogenated aromatic compounds occurring as environmental pollutants is of increasing public health concern. This newly developed human EROD bioassay represents a novel tool for assessing the effects of such pollutants on human health. Furthermore, it facilitates study of the more fundamental aspects of pollutant-mediated toxicity such as those addressed in this study. The numerous interactions that may potentially occur among constituents of a complex pollutant mixture indicate that the ultimate toxic effects of a mixture would be highly case-specific. In order to begin to address the many possible interactions within a mixture, rapid and sensitive screening methods will be needed. The HepG2 EROD bioassay represents such a technique which has the addition advantage of being directly applicable to studies of pollutant effects on human health.

Results of the present study demonstrate that pronounced interactions may occur in mixtures of polyhalogenated xenobiotics. PCB #77, for example, appears to be capable of competing effectively with TCDD for *Ah* receptor-mediated CYP IA1 induction without being an effective agonist on its own. This suggests that similar interactions in a complex mixture would result in "masking" toxicity of agonists (like TCDD) by non-agonists such as PCB #77. Therefore, if such mixtures are assessed for TCDD toxic equivalency in the murine CYP IA1 bioassay[2], for example, the observed CYP induction would lead to an underestimation of the true toxic potency of the mixture. Similarly, if constituents such as PCB #126 are present, which are not only effective agonists but are competitors for the *Ah* receptor, the toxicity of both of the competing agonists would be masked. Research is ongoing with respect to other such interactions which may occur within complex pollutant mixtures containing polycyclic and polyhalogenated aromatic compounds.

References

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