IMPLICATIONS FOR DIETARY INTERACTIONS OF TEQs AND ENDOGENOUS/PHYTOCHEMICAL Ah RECEPTOR LIGANDS

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Introduction

The aryl hydrocarbon receptor (AhR) was initially identified as the intracellular target for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related toxic halogenated aromatic hydrocarbons [1]. Different structural classes of polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) have been investigated to determine their activities as AhR agonists [2-4]. PCDDs and PCDFs which exhibit high binding affinity for the AhR contain four lateral (2, 3, 7 and 8) chlorine substituents; the most active PCBs are substituted in both *para* and two or more *meta* positions (Fig. 1). These results were consistent with a hypothesis that the AhR bound with high affinity to coplanar isosteric halogenated aromatic compounds with dimensions (3 x 10 °A) similar to that of TCDD [2]. Halogenated aromatic compounds such as the mono*ortho*-substituted PCBs which deviated from these ideal dimensions and coplanarity typically exhibit lower binding affinities for the AhR. Several studies also show a rank order correlation between structure-AhR binding vs. structure-toxicity relationships among halogenated aromatics, and these observations supported a role for the AhR in mediating the toxic effects induced by these compounds [2-5]. This was subsequently confirmed in studies with AhR knockout mice which are resistant to TCDD-induced biochemical and toxic responses [6, 7].

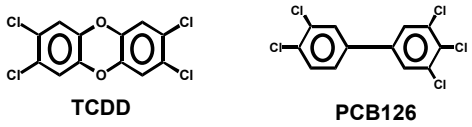

Figure 1. Toxic halogenated aromatics.

Toxic Equivalents: Mechanism-Based Risk Assessment

Halogenated aromatic compounds are industrial and combustion by-products that have been identified in every component of the ecosystem, and there is particular concern regarding the adverse environmental and human health impacts of TCDD and other AhR-active compounds [8]. Initial hazard and risk assessment of halogenated aromatics primarily focused only on levels of TCDD, even though environmental samples contained complex mixtures of PCDDs, PCDFs and PCBs. AhR-dependent *in vivo* or *in vitro* bioassays clearly demonstrated that the potency of a mixture of halogenated aromatics was dependent on all the AhR agonists in the mixture [9-12]. These observations, coupled with the common AhR-mediated mechanism of action for TCDD and related compounds led to development of the toxic (or dioxin) equivalents (TEQs) method for hazard and risk assessment of TCDD and structurally-related compounds [13-16]. The TEQs of any mixture are equal to the sum of the concentration of individual (i) congeners times their potencies (TEF_i) relative to TCDD (TEF = 1.0).

$$TEQ = \sum[PCDD_i] \times\cdot TEF_i + \sum[PCDF_i] \times TEF_i + \sum[PCB_i] \times TEF_i$$

TEF values have been developed for PCDDs and PCDFs, and these are commonly used for risk assessment of mixtures containing these compounds. TEFs for PCBs have also been proposed [16]

and their contribution to the TEQs of some environmental samples and food extracts can be substantial.

Problems with the TEQ/TEF Approach for Risk Assessment

Initial studies using the TEF approach for mixtures confirmed that the calculated TEQ values for a PCDD/PCDF mixture were comparable to the observed toxicities [9-12]. In contrast, there are several reports that show non-additive interactions between AhR-active and weakly active/inactive compounds, particularly among the PCBs (reviewed in [17, 18]. Several studies show that 2,2',4,4',5,5'-hexachlorobiphenyl (PCB #153) antagonizes TCDD- or 3,3',4,4',5-pentachloro-biphenyl-induced biochemical and toxic responses. Moreover, the ratio of antagonist/agonist required for inhibitory responses (usually > 1,000/1) are observed in many environmental mixtures. While these observations do not negate the use of the TEF/TEQ approach for risk assessment of halogenated aromatics, it is highly probably that for some mixtures this method will overestimate toxicity due to antagonist interactions.

Most early studies on the AhR and other ligand-activated receptors focused on characterizing receptor-ligand interactions among narrowly defined chemical classes such as the halogenated aromatics (AhR) and steroid hormones (for steroid hormone receptors, SHRs). However, it is now apparent that the AhR and SHRs are highly promiscuous and bind with variable affinities to structurally-diverse chemicals (reviewed in [19-21]). Currently, there is concern by scientists and regulatory agencies regarding the potential adverse effects of synthetic estrogens (xenoestrogens) and phytoestrogens on decreased male reproductive capacity and breast cancer [22]. Most of the compounds with a hypothesized link to these problems are chemicals which exhibit low to moderate binding of affinity for the estrogen receptor. There have been an increasing number of reports showing that structurally-diverse synthetic compounds, naturally-occurring phyto-chemicals, and endogenous biochemicals also bind the AhR with low to moderate affinities [19, 20]. Some of these classes of compounds include polynuclear aromatic hydrocarbons, aromatic amines, indole-3-carbinol and tryptophan-derived compounds, other heterocyclic aromatic hydrocarbons, structurally-diverse pesticides and drugs, bilirubin and biliverdin, resveratrol, steroids/sterols, carotenoids, and bioflavonoids [22-36] (Fig. 2). AhR binding affinities of these ligands are variable, and some of these compounds can exhibit both AhR agonist and/or antagonist activities depending on their concentrations and cell context. Some of these chemicals including indole-3-carbinol and related compounds and bioflavonoids are present in high concentrations in the human diet and therefore could significantly modulate the potential impacts of halogenated aromatics (TEQs) [17, 18, 36]. For example, daily intakes of TEQs primarily as trace contaminants of food have been steadily decreasing and are in the 50 - 200 pg range and serum TEQs are approxi-mately 0.1 pM [37]. In contrast, serum levels of flavonoids that generally exhibit AhR antagonist activities at concentrations < 1.0 μM are in the nM to low μM range. Thus, serum ratios of flavonoids/TEQs-TCDD are 10^4 - 10^6, and these ratios are comparable to those required for antagonism of TCDD-induced responses by some phytochemicals [25-27, 32, 36]. 7-Ketocholesterol is also an AhR antagonist with a competitive binding IC_{50} value of 500 nM [33]. Plasma concentrations of

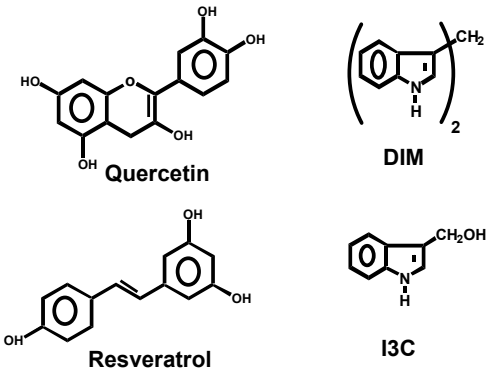

Figure 2. Chemoprotective phytochemicals.

7-ketocholesterol range from 20 to 200 nM in healthy humans [38], suggesting a potential protective effect against dietary TEQs.

Summary
Humans are exposed to a bewildering number of AhR-active chemicals in the diet which exhibit both agonist and antagonist activities. TEQs for TCDD-like halogenated aromatics are relatively low in terms of intake and serum levels. In contrast, dietary intakes of phytochemical AhR agonists are high. It can be argued that interactions between phytochemicals and TEQs (TCDD) may not be important due to rapid metabolism and clearance of the former compounds. However, there are relatively high serum concentrations of phytochemical/sterol AhR antagonists and therefore the potential for tissue-specific chemoprotective interactions should be further investigated.

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