

## TYPES OF AhREs AND THEIR IMPLICATIONS

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### Introduction

In their insightful 1982 review, Poland and Knutson<sup>1</sup> compared the polycyclic aromatic hydrocarbons (PAHs) and halogenated aromatic compounds (PHAHs):

While both classes of compounds bind to the [AhR] cytosol receptor and induce AHH activity, the characteristic spectrum of toxic responses are only observed with halogenated compounds. If toxicity is mediated through the receptor, one must explain this discrepancy. One might postulate that enzyme induction is an early event, but that toxicity (especially epithelial cell proliferation and metaplasia) is a much later event and requires persistent receptor occupation and gene expression [p. 539]

Riddick et al. showed that while 3-methylcholanthrene (3-MC) had an affinity for mouse AhR about 3-4 times less than 2,3,7,8-TCDD, it was about 1000 times less potent at inducing CYP1A1 aryl hydrocarbon hydroxylase activity as assessed after 14 hours.<sup>2</sup> This difference was due at least in part to the more rapid metabolism of 3-MC.

According to this view, the extraordinary biologic potency of TCDD is due to its high affinity for the AhR and its resistance to metabolism. This idea appears to dominate thinking in the dioxin field as does the metaphor of the agonist-receptor pair as a key and lock: if the key fits the lock, the lock opens.

### Types of AhREs

Recent data argue that these ideas are not sufficient: more than AhR affinity and half-life are involved. Matikainen et al. showed that 9,10-dimethylbenzanthracene (DMBA), a prototype PAH, induced *Bax* expression and oocyte apoptosis in mouse ovaries.<sup>3</sup> The CYP1B1-derived metabolite DMBA-3,4-dihydrodiol (DMBA-DHD) had the same effect. Both DMBA and DMBA-DHD are AhR agonists. The effects of DMBA-DHD were blocked by the AhR antagonist  $\alpha$ -naphthoflavone and did not occur in AhR<sup>-/-</sup> mice. A *Bax* promoter-green fluorescent protein (GFP) construct was then microinjected into mouse oocytes. High GFP was seen in the oocytes exposed to DMBA-DHD, but not in the following cases: oocytes treated with vehicle, reporter construct missing the *Bax* promoter, *Bax* promoter inactivated due to mutation of the AhRE core sequence (Figure 1), or when oocytes were AhR<sup>-/-</sup>. These data indicate that the effects are AhR mediated. Other experiments showed the causal role of *Bax* in the apoptosis of the oocytes.

But there was a surprise: TCDD neither destroyed oocytes nor effectively induced *Bax* above controls. Yet TCDD and PAHs both induce ovarian P450 enzymes. The *Bax* promoter has two core AhR responsive elements (AhREs): See Figure 1 (adapted from Matikainen et al.<sup>3</sup>). The *Bax* promoter contains a guanine or cytosine in the +3 position (underlined) downstream of the core sequences. Earlier work indicated that the *CYP1A1* promoter must have an adenine in this position to be active in a reporter assay after exposure to TCDD.<sup>4,5</sup> When Matikainen et al. mutated these two bases of the *Bax* promoter to adenine (Figure 1), TCDD was able to effectively induce *Bax*.

Figure 1. AhRE sequences of the wildtype, mutated and inactivated *Bax* promoter. Core sequences are indicated in bold. As shown by Matikainen et al.,<sup>3</sup> the wildtype was responsive to DMBA but not TCDD. Mutation of key flanking nucleotides (underlined) to the pattern found in *CYP1A1* made the promoter responsive to TCDD. Mutation of nucleotides in the core sequence (italics) inactivated the promoter.



### Research Implications

These experiments explain why DMBA—but not TCDD—induce *Bax* and oocyte apoptosis in the mouse ovary. But they have, we believe, much wider implications. In particular, the lock and key metaphor does not tell the whole story. It is not just the ligand and receptor—or the binding affinity and half life—that matter but also the type of AhRE. Type 1 AhREs, as found in *CYP1A1*, have an adenine in the +3 flanking position and are activated by both TCDD and DMBA (and other PHAHs/PAHs). Type 2 AhREs, as found in *Bax*, have a G or C in the +3 flanking position and are responsive to DMBA but not TCDD.

These findings suggests several research questions:

- What is the molecular basis for the difference in interaction of Type 1 and 2 AhREs with AhR-ARNT bound to TCDD or DMBA?

- Matikainen et al. examined TCDD and DMBA/DMBA-DHD. Other AhR ligands—PHAH, PAH, others—should also be screened for activity with reporter assays for the type 2 (*Bax*-like) AhRE. Such assays may provide a way to divide AhR ligands into different functional classes.
- Which genes have type 1 and type 2 AhREs? Glutathione S-transferase Ya, rat and human P4501A1 have type 1 AhRE sequences; quinone reductase has T instead of A at the +3 position.<sup>6</sup> Experiments could proceed in several ways, for example: scanning the genome for type 1 and 2 sequences and then test functionality; comparing gene arrays after exposure to TCDD or DMBA.
- Could type 2 AhREs explain other anomalous effects? For example, DMBA and DMBA-DHD but not TCDD cause apoptosis of pre-B cells via a mechanism that is at least partly AhR mediated.<sup>7</sup>
- Type 1 AhREs respond to PHAHs and PAHs while type 2 AhREs respond to DMBA (and presumably other ligands). However, dioxin-like compounds cause some effects that PAHs don't. If there are only two types of AhREs, then the persistence of TCDD (and other PHAHs) retains its central importance. But there is another possibility: Could there be a type 3 AhRE, responsive to dioxin-like compounds but not PAHs?

### AhRE types and TEFs

Many PAHs are AhR ligands and exhibit some AhR-associated biochemical effects, e.g., CYP1A1 induction. One estimate of total human exposure to AhR agonists suggested that PAHs and certain plant-derived compounds were more important than the PCDDs, PCDFs, and dioxin-like PCBs.<sup>8</sup> Others argued against this position largely on pharmacokinetic grounds.<sup>9</sup> PAHs are not included in the official TEF schemes. A recent USEPA review rejected application of TEFs to PAHs for several reasons including: short half-lives, uncertainty regarding the role of the AhR in their toxicity, and differences in their toxicity, e.g., mutagenicity of reactive PAH metabolites.<sup>10</sup> There is now another reason to reject application of TEFs to PAHs: at least one kind of AhR-mediated toxicity of DMBA (oocyte apoptosis) is not caused by TCDD because of the existence AhRE types.

TEFs are currently applied to PCDDs, PCDFs and certain PCBs.<sup>11</sup> Does the discovery of AhRE types mean that TEFs should not be applied to even this restricted group of chemicals? The molecular mechanisms of most effects of dioxin are not known, much less the AhRE types of the genes involved in these effects. TEFs are used in a science-based regulatory context with a need to make decisions without having to re-examine the TCDD toxicology for every candidate. According to a World Health Organization expert committee,<sup>11</sup> a compound must meet certain criteria to be included in the TEF scheme:

- 1) “show a structural relationship to the PCDDs and PCDFs”
- 2) “bind to the Ah receptor”
- 3) “elicit Ah receptor-mediated biochemical and toxic responses”
- 4) “be persistent and accumulate in the food chain”

Although AhRE types add uncertainty—“toxic responses” in the third criterion should read dioxin-like toxic responses—the WHO criteria appear reasonable. It may also be possible to construct a new type of TEF for Type 2 AhRE-ligands.

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