Estrogen and Dioxin Receptors
Or,
The World's Most Powerful Compound *vs.* The World's Most Toxic Compound

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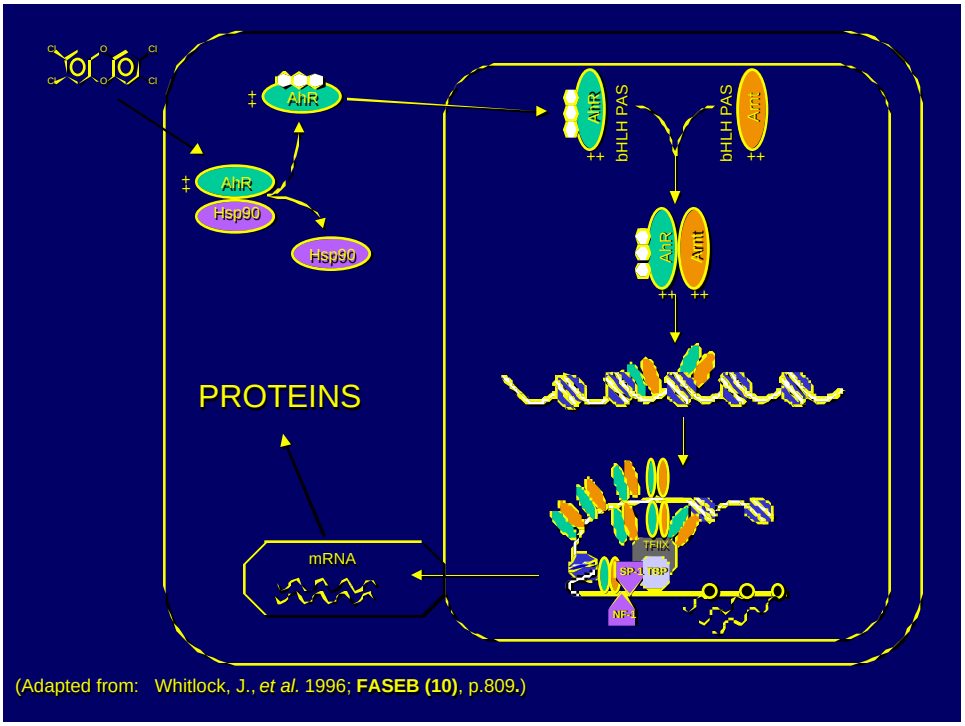
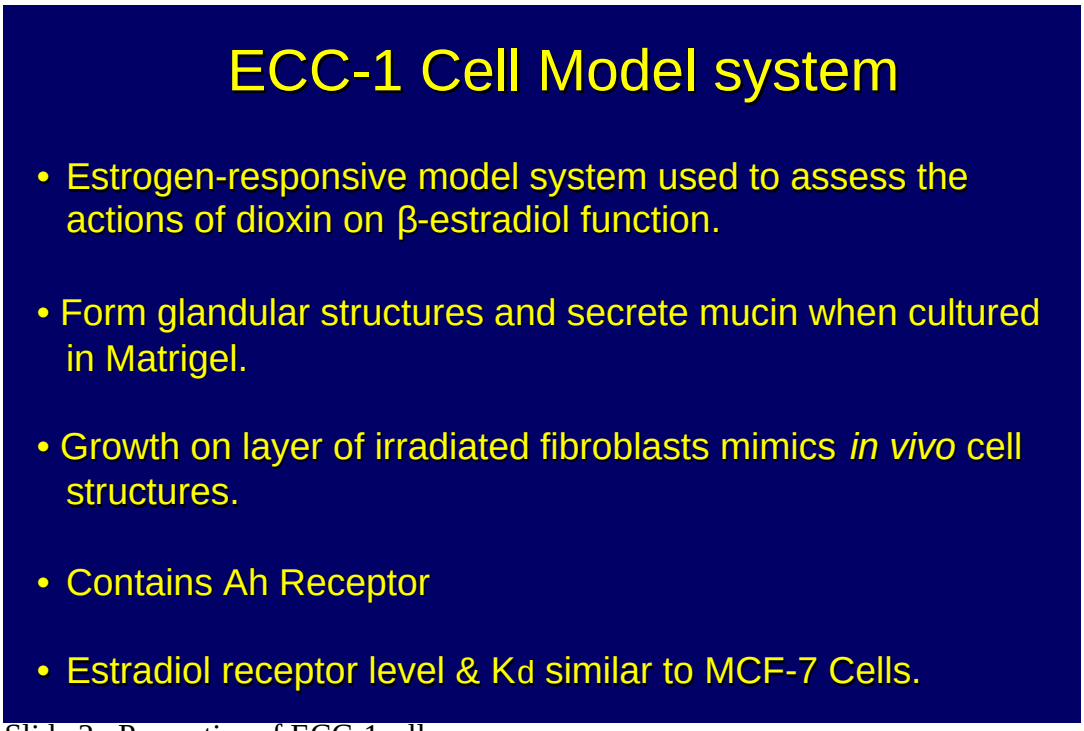


Slide 1. Dioxins are ubiquitous environmental contaminants that constitute a family of some 75 related polychlorinated dibenzoparadioxins. These compounds exert their toxicity *via* the Ah receptor independently discovered some thirty years ago by Alan Poland and Daniel Nebert. The receptor is an intracellular receptor of the basic helix-loop-helix family that exists in the unbound state as a heteromer. Once ligand is bound to receptor, it complexes with other proteins within the nucleus, binds to DNA at specific sites known as AhREs (formerly known as XRE, or DRE) to activate transcription of many proteins, including three members of the cytochrome P450 super family, CYP1A1, CYP1A2, and CYP1B1. The best ligands for the Ah receptor are planar

aromatic compounds that have at least three of the four lateral positions occupied by a halogen, and that fit into a theoretical rectangle of 8 x 14 Angstroms.

Dioxins are implicated in endometrial disease. We selected the ECC-1 cell line developed by the late Dr. P. G. Satyaswaroop of the Hershey Medical Center, Penn State University. The properties of this cell line are given in the second slide. This cell line is an excellent model system in which to study the action of dioxins on estrogen and to elucidate the mechanism of dioxin-induced endoometriosis.

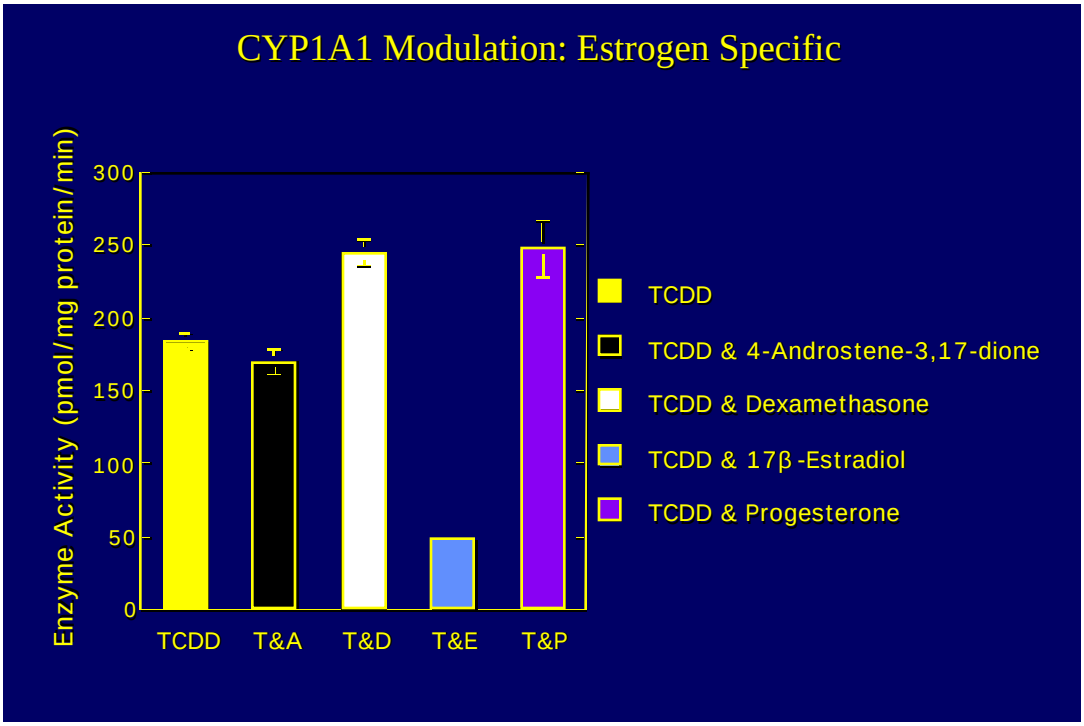


Slide 2. Properties of ECC-1cells.

Dioxins alter estrogen action in these cells. Exposure of these to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the archetypical ligand for the Ah receptor and 2,3,7,8-tetrachlorodibenzofuran (TCDBF), a partial agonist for the Ah receptor, of ECC-1 cells showed a concentration, and time dependent induction of cytochrome P450 1A1 (CYP1A1). For more information, see (Ricci et al.,

1999). We asked the question of whether estrogen reciprocally altered the action of TCDD, using CYP1A1 induction as the measure of estradiol action. ECC-1 cells exposed to dioxin and 17β-estradiol showed a concentration and time-dependent decrease in CYP1A1 activity that was reversed by simultaneous exposure to 4-hydroxytamoxifen and ICI 182-780, antagonists for the estrogen receptor. Estradiol affects CYP1A1 at the level of messenger RNA. We demonstrated decreases in CYP1A1 mRNA in nuclei obtained from cells exposed to TCDD, and 17 β -estradiol. This effect was reversed by exposure to 4-hydroxytamoxifen and ICI 182-780. This decrease in Ah receptor action was specific to the estrogen receptor:

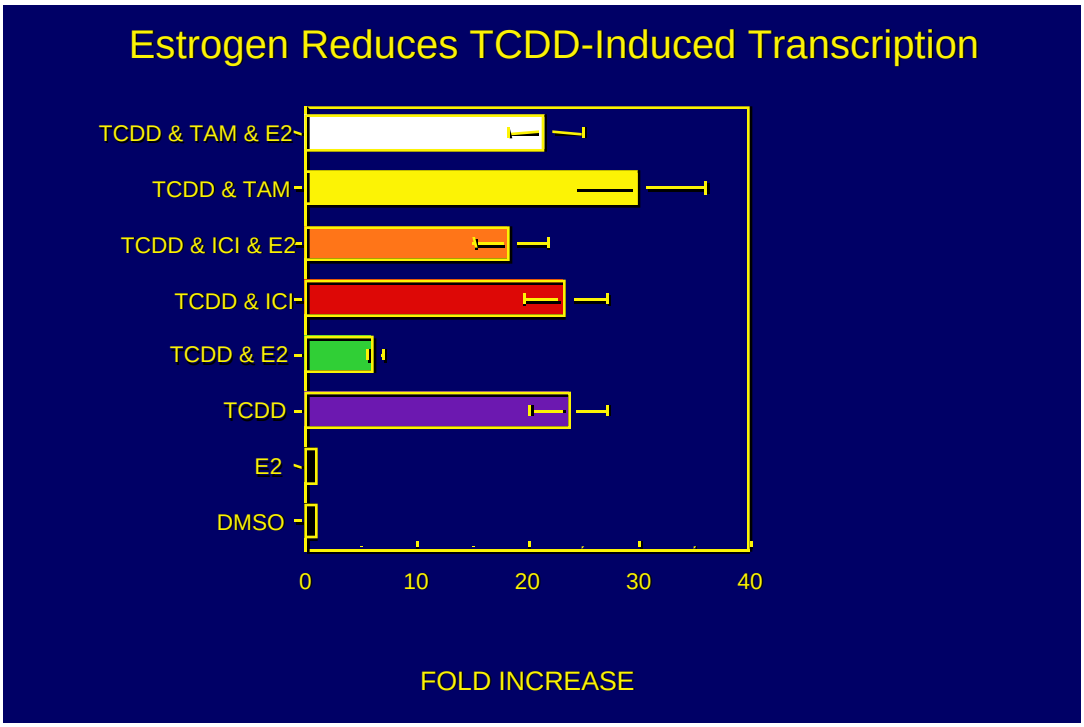
In the following slide, we show CYP1A1 activity in ECC-1 cells exposed to TCDD alone, TCDD and 4-androstene3,17-dione, TCDD plus dexamethasone, an agonist of the glucocorticoid receptor, TCDD in the presence of 17β-estradiol, and TCDD and progesterone. Only in the presence of 17β-estradiol was the induction of CYP1A1 decreased.



Slide 3. Decrease of Ah receptor action is estrogen specific.

The action of estradiol is at the level of transcription. We examined the action of 17β-estradiol on the transcription of the CYP1A1 gene by nuclear runoff showed that 17β-estradiol altered the rate

of transcription of the gene, furthermore the action of 17β-estradiol was reversed by 4-hydroxytamoxifen and ICI 182-780. We transfected ECC-1 cells with the cyp rpomoter attached to the luciferase gene, which was also inhibited by 17β-estradiol, and transcription activity was restored by estrogen antagonists.

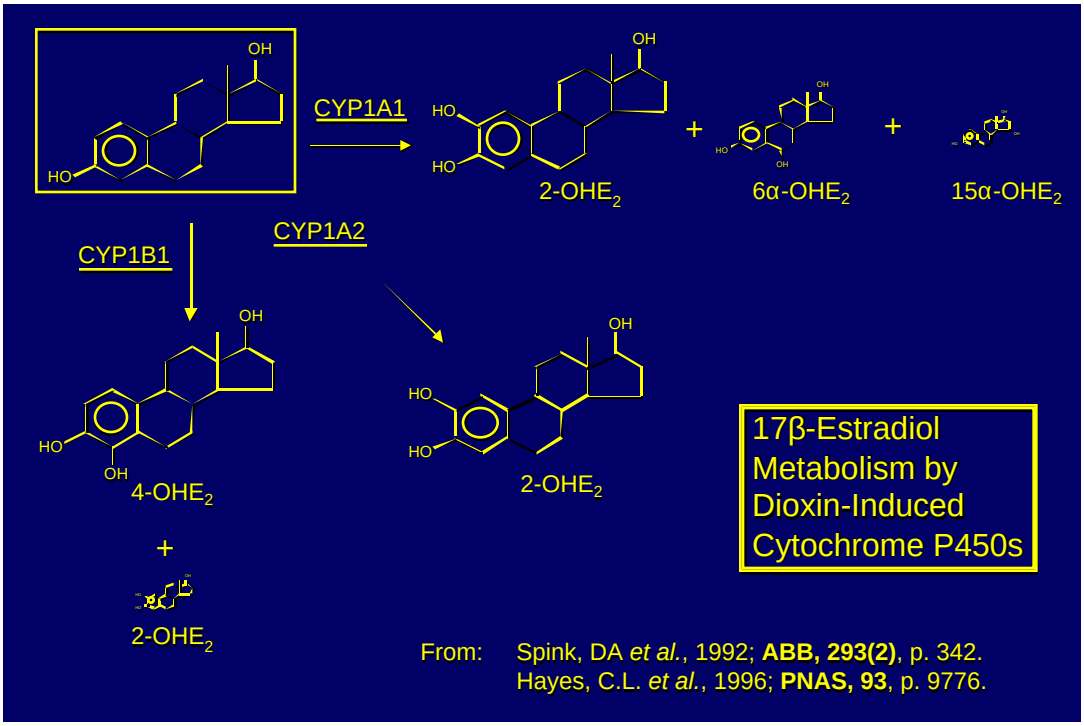


Slide 4. 17β-estradiol reduced TCDD-induced transcription of the CYP1A1 gene.

Estradiol inhibition of CYP1A1 induction was cell specific. We examined the action of 17β-estradiol of TCDD-induction of Cyp1A1 in MCF-7, ECC-1, HuE keratinocytes, and HEP3B liver cells to determine whether this effect was specific to hormone-regulated cells. Our data showed no effect on TCDD-induction of CYP1A1 in liver or keratinocyte cells, which showed specificity of the action of 17β-estradiol to hormone-regulated cells.

Two cytochromes are important in 17β-estradiol metabolism in endometrium, CYP1A1 and CYP1B1. The action of 17β-estradiol was specific to CYP1A1, but had no effect on CYP1B1. We postulated that this results from ER binding NF-1 a co-transcription factor in nucleus. Transfecting cells with NF-1 restored the ability of TCDD to overcome inhibition by 17β-estradiol. ER is present in these cells in a ratio of100:1 greater than the Ah Receptor. CYP1B1 promoter

does not contain binding sites for NF-1, but CYP1A1 does. These data demonstrate the reason that the 4-catechol derivative of 17β-estradiol is the major metabolite in endometrium.



Slide 5. Metabolic scheme of 17β-estradiol in endometrium.

Our working model is that in the presence of high levels of estrogen, CYP1A1 activity is diminished, but CYP1B1 is fully active. 4-OHE2 is the major metabolite of CYP1B1 activity, and is the major metabolite found in endometrium. This derivative of estradiol is thought to be more toxic than the 2-OHE2 derivative catalyzed by CYP1A1.

References:

Ricci, M. S., Toscano, D. G., Mattingly, C. J., and Toscano, W. A., Jr. (1999). Reversible Modulation of Ah Receptor Action by 17β-Estradiol in Cultured Human Endometrial Cells. J. Biol. Chem. *274*, 3430-3438.

Ricci, M. S., Toscano, D. G., and Toscano, W. A., Jr. (1999). ECC-1 Human Endometrial Cells As A Model System to Study Dioxin-mediated Steroid Hormone Disruption. In Vitro Cell Dev. Biol. *35*, 183-189.