

Estrogen Receptor Project

E. Glustrom '07

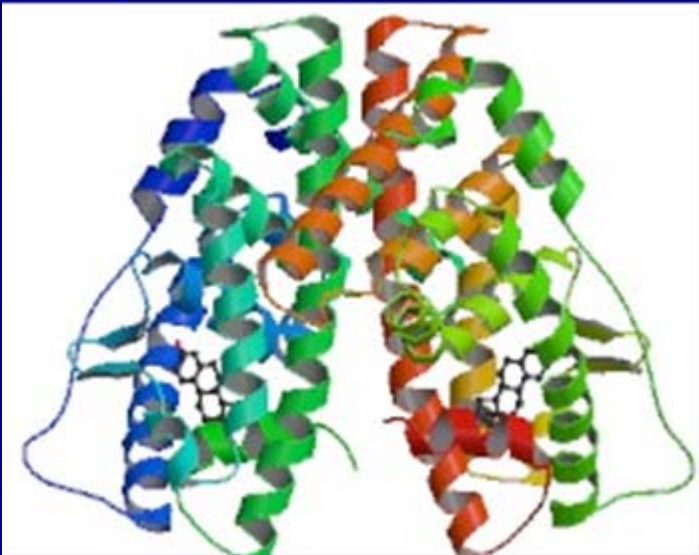
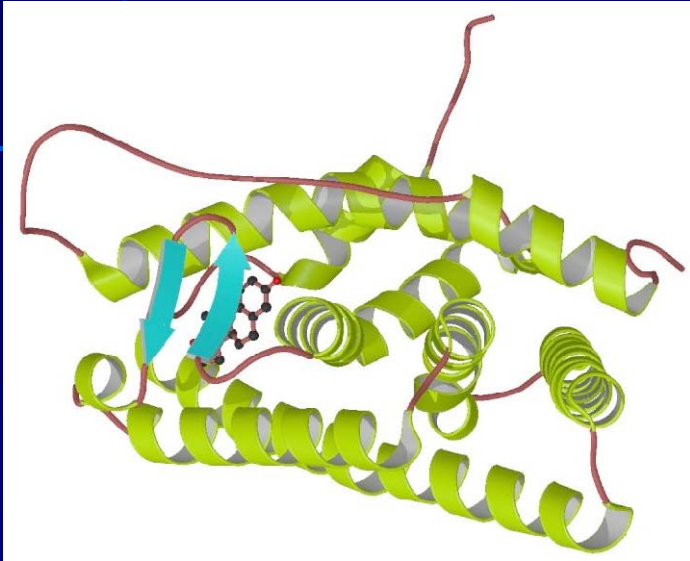
Beckman Scholar 2006-2007



FOUNDATION

- Manmade materials such as pesticides and their derivatives have been implicated in human pathologies such as cancer and autism.
- *Can these compounds bind to and interfere with steroid receptors such as the estrogen receptor? Are the **xenoestrogens**?*
- We'll use polarization anisotropy to test.

Estrogen Receptor alpha



- Ligand binding domain, DNA binding domain, 2 transcription activating domains
- Two wedges, each of 12 alpha helices, form a dimer with the LBD in between at the narrow end
- Can bind to a range of ligands because the LBD is so large
- ER + ligand moves from cytosol to nucleus and binds to the estrogen response element (ERE) section of DNA

http://www.ebi.ac.uk/msd-srv/msdtarget/strs_images/1qkt.jpeg

http://www.pdb.org/pdb/images/1g50_bio_r_250.jpg?bioNum=2

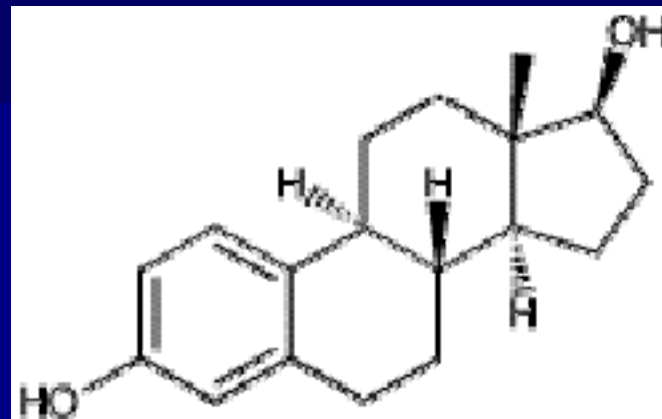
Can Xenoestrogens mimic estrogen?

Introduction

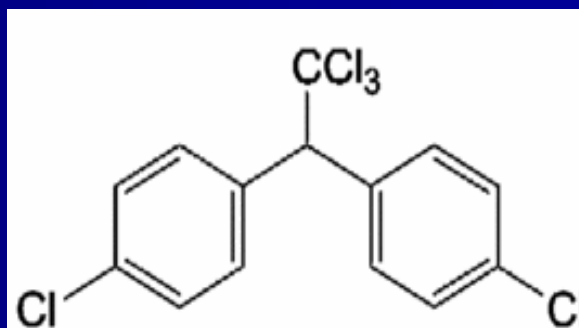
DDT was banned in 1972 due to harmful effects the powerful insecticide was having on wildlife. Since its ban in 1972, evidence has been found that DDT and its hydroxylated metabolites may mimic estrogen. DDT has been largely replaced by methoxychlor. Methoxychlor is similarly broken down into hydroxylated metabolites that are potentially estrogenic.



A phenolic ring is the primary structural feature necessary to bind the estrogen receptor. Thus, the present research hypothesizes that the phenolic metabolites, but not the chlorinated insecticides, will be estrogenic.



Estradiol



DDT

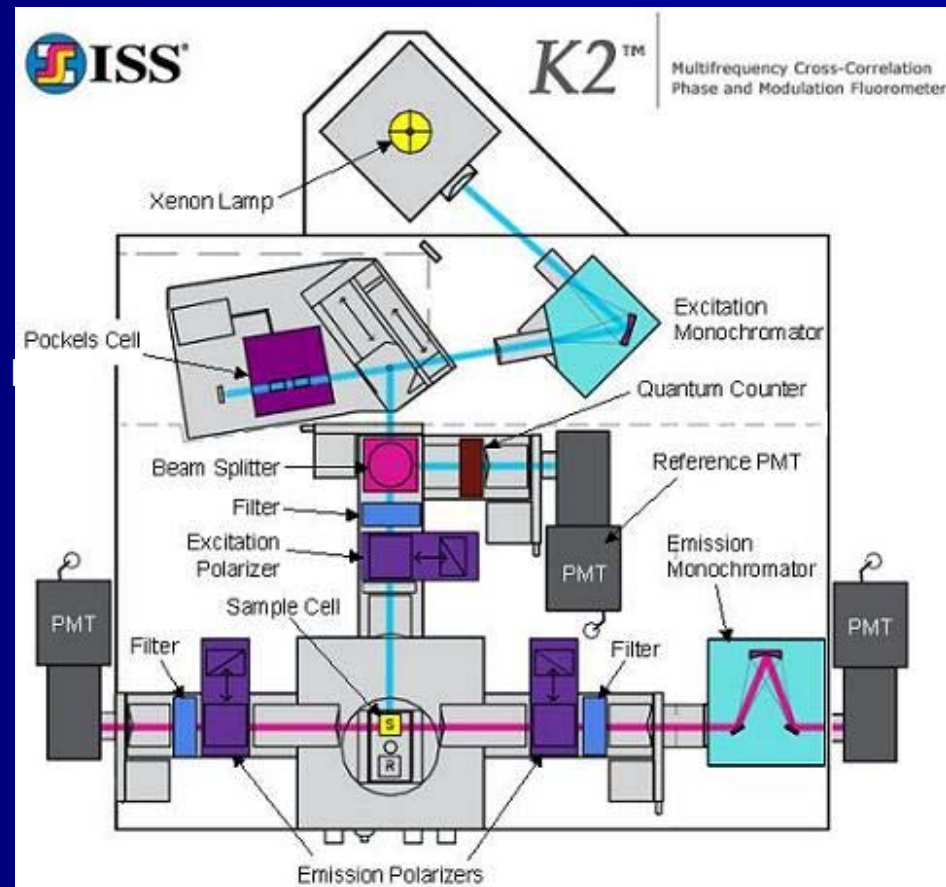
Can Xenoestrogens mimic estrogen?

Methods

In order to determine the estrogenicity of DDT, methoxychlor, and their metabolites a competitive binding assay was performed using estrogen receptor (ER) and a fluorescent estrogen analogue. In addition, the ability of the insecticides and their metabolites to initiate estrous behavior in ovariectomized female rats was measured to determine the *in vivo* estrogenic effect of the compounds.

Can Xenoestrogens mimic estrogen? *in vitro* biochemical assays

- Polarization Values were measured using a ISS K2 multifrequency phase fluorometer

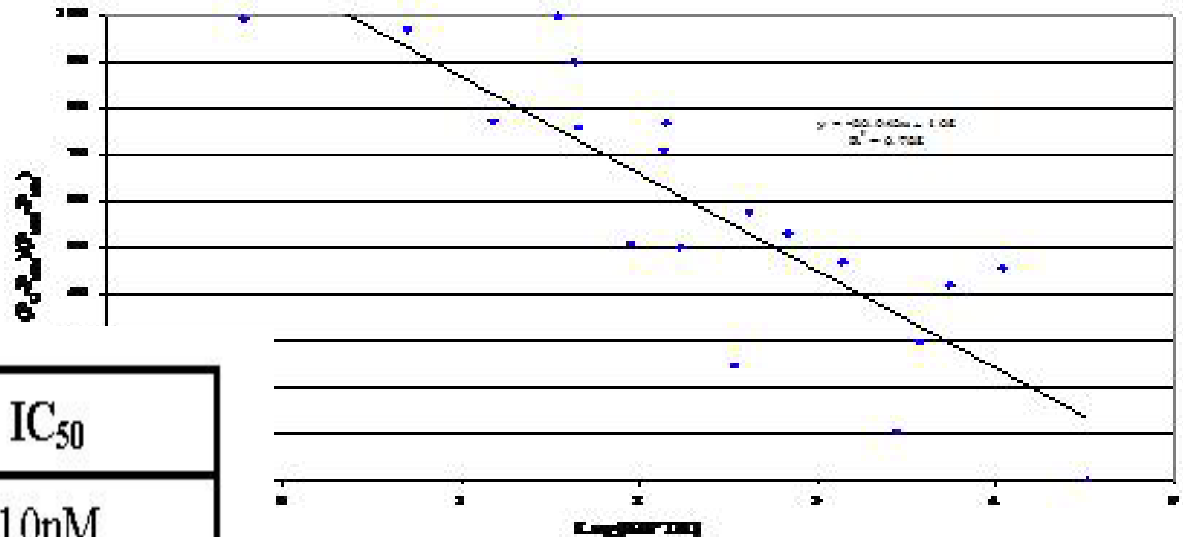


Can Xenoestrogens mimic estrogen?

in vitro biochemical assays

Competitive Binding Assay - Measures polarization versus log Conc of Competitor

HPTE Competitive Binding with ER

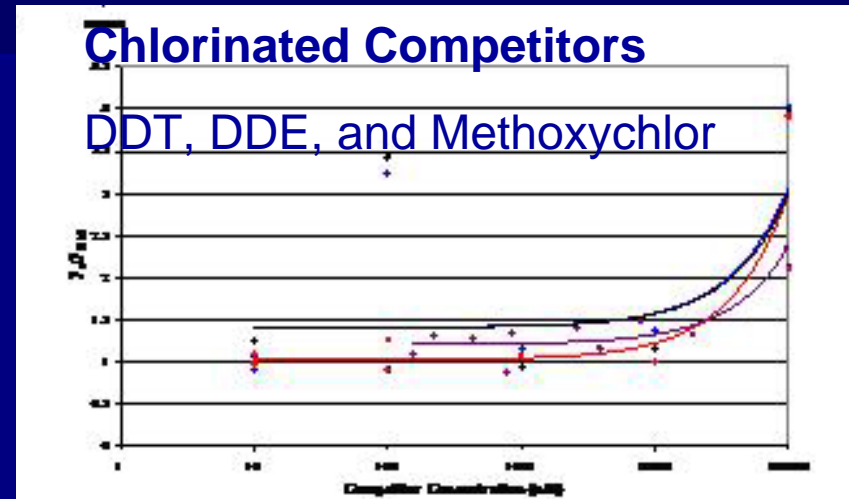
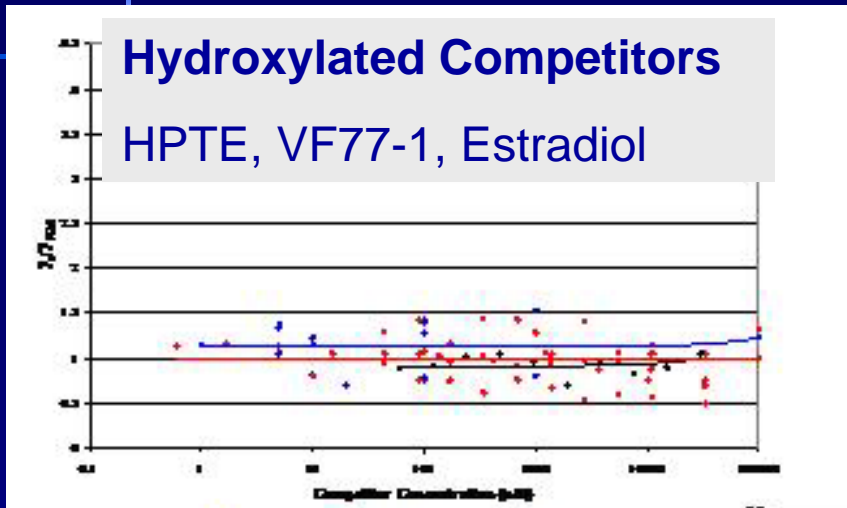


Compound	IC ₅₀
Estradiol	10nM
HPTE	588nM
VF 77-1	310nM
VF 72-1	880nM

IC₅₀ values of displacement of fluormone from ER I

Can Xenoestrogens mimic estrogen?

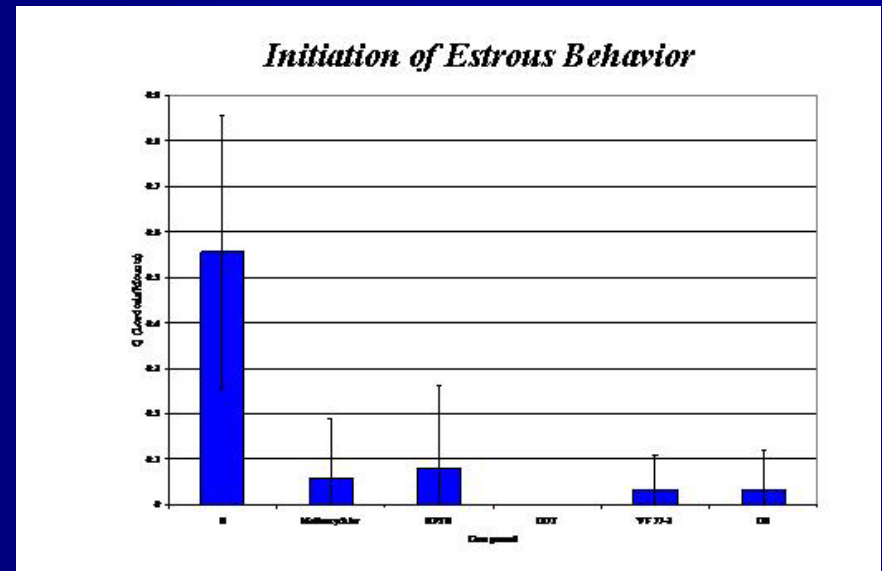
in vitro biochemical assays



- Pesticides and derivatives caused precipitation of the ER at concentrations well below their solubility limits.
- Chlorinated compounds much more effective at denaturing ER than hydroxylated

Can Xenoestrogens mimic estrogen? *in vivo behavioral assays*

- In vivo assays- the ability of the DDT metabolites or pesticides to initiate estrous behavior in ovariectomized rats was measured.
- Only four compounds caused any recovery of estrous behavior:



Can Xenoestrogens mimic estrogen?

■ Conclusions

- only the hydroxylated derivatives of DDT (VF77-1) and methoxychlor (HPTE) bind significantly to the ER *in vitro*.
- Only minimal initiation of estrous behavior was observed when pesticides or their derivatives administered *in vivo*.
- Methoxychlor is NOT a safe alternative to DDT.

■ Future work

- Dosage curves should be determined for in vivo administration of pesticides
- Other candidates with appropriately placed hydroxyl groups should be tested.

Xenoestrogens mimic estrogen

IC₅₀ Values of ER Competitors

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Estradiol

<http://www.3dchem.com/imagesofmolecules/Estradiol.gif>

http://courses.washington.edu/z490/ed/Other%20Risks%20for%20People_files/image002.gif