Manmade materials such as pesticides and plasticizers 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
Xenoestrogens mimic estrogen

- Estradiol - primary biological substrate
- Xenoestrogens have the common feature of an aromatic ring with an electronegative atom on the ring (DDT and bisphenol A)
Pyrethrins - are they xenoestrogens?

- What are they?
  - Naturally occurring pesticide found in chrysanthemum flowers
  - Pyr and their derivatives are found in many commercial products, including household insecticides, animal sprays, lice treatment shampoos and mosquito repellants

- Pyrethrin I: $R = H$
- Pyrethrin II: $R = CO_2$
- Permethrin
- Bifenthrin
- Fenvlralete
- Acrinathrin

DDT
Fluorescence Polarization

Fluorescent Lifetime = $\tau_{fl}$

Rotational correlation time = $\theta_{rot}$

- If $\tau_{fl} << \theta_{rot}$ (bound fluoromone) molecule doesn’t rotate during excited state lifetime and emitted light will be polarized.
- If $\tau_{fl} >> \theta_{rot}$ (free fluoromone) molecule doesn’t rotate during excited state lifetime and emitted light will be depolarized.
- A fluorescent tagged molecule is displaced from the receptor by a competitor molecule (estrogen or xenoestrogen)
- When this occurs, the released tagged molecule rotates more rapidly in solution, and thus polarization decreases.

http://www.iss.com/resources/tech2/
Xenoestrogen Project

![Fluormone and Estrogen or Pyrethroid Diagram]

- **Fluormone**
- **Estrogen or Pyrethroid**

Graphical representation showing the interaction between fluormone and estrogen or pyrethroid over time (ns) and excitation light polarization.
Polarization Measures Binding

Increasing amounts of estrogen receptor

When small molecules which tumble freely (low polarization) bind to much larger molecules which don’t tumble as easily (high polarization), there is a concomitant increase in polarization.

5 nM estrogen analogue fluormone

Fluormone binding to Estrogen Receptor
Pyrethrins tested ARE able to mimic estrogen in the displacement of fluormone from the ER.