

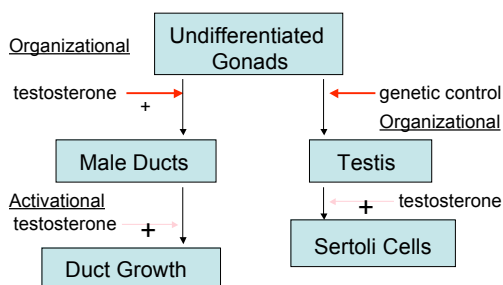
ES/RP 531
Fundamentals of Environmental Toxicology

Lecture 8
Endocrine System Toxicity
Part 2: In Vitro & In Vivo Tests

Mechanisms of Endocrine
Disruption

- Organization vs. Activation Model
 - Organizational effects occur early in an individual's lifetime during critical periods and induce permanent effects
 - Activational effects usually are transitory actions occurring during adulthood

Organization Vs. Activation Model



Organization vs. Activation Model

- Embryos particularly vulnerable
 - Critical periods of sensitivity during embryonic organization
 - Potential storage of lipophilic persistent contaminants in yolk or fat reserves
 - Free circulating contaminant may be greater than bound contaminant

Organization/Activation Effects of HAAs

Species	Organizational Effect	Activational Effect	HAAs
Salmon	premature sex. maturity	fertility decrease	PCBs, DDT, dioxins
Alligator	testosterone down	poor quality eggs	DDE
Bald Eagle	embryonic mortality & deformities	fertility down	PCBs, DDE, Dieldrin

Guillette (1995) EHP 103:157

Suspected Hormonally Active Agents

- Pesticides
 - DDT, DDE
 - Vinclozolin fungicide
 - Methoxychlor insecticide
 - Atrazine
- PCBs (polychlorinated biphenyls)
- PAH (polyaromatic hydrocarbons)
- Dioxins (TCDD)
- Bisphenol A
- Nonylphenol
- Tributyl tin fungicide
- Plasticizers (phthalates)
- Metals (Pb, Hg)
- Benzophenone derivatives
- PBDEs (polybrominated diphenyl ethers)
- Perchlorate
- Natural biochemicals (many flavonoid antioxidants)

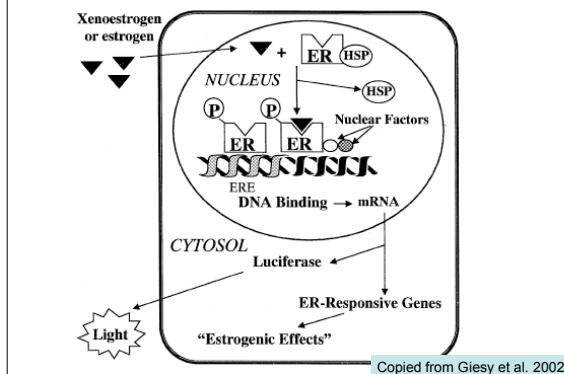
In-vitro Effects: Estrogen Agonists & Antagonists

- Biochemical Screening Tools
 - E-Screen (Soto et al.)
 - Yeast Cell Assays (McLachlan, Sumpter et al.)
 - Competitive Binding Assays (many)
 - Vitellogenin Assay (Sumpter et al.)
 - Androgen Receptor Assay (Gray, Kelce et al.)
- Extraordinary Range of Sensitivity (from pM to μ M)
- Anti-Estrogenic Effects [PCBs, Dioxins] (Safe et al.)

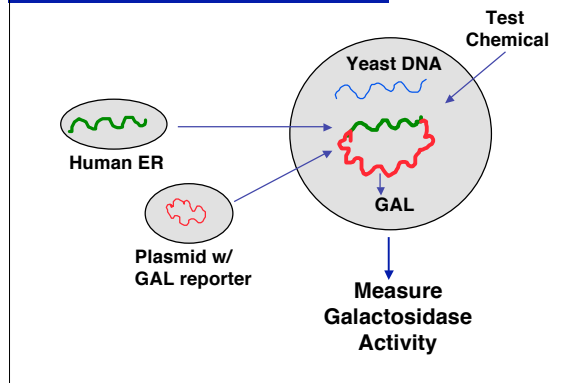
Testing for HAA Activity

- In vitro tests
 - YES screen (galactosidase reporter gene)
 - Anti-androgenic screen (luciferase reporter gene)
 - E screen (MCF-7 cell line)

Biochemical Basis for In-Vitro Screens

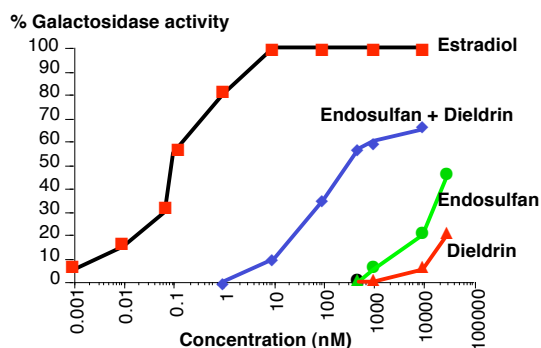


Yeast Estrogen System



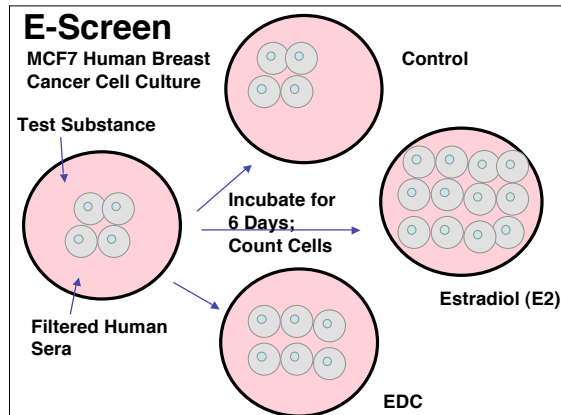
Endocrine Disrupter Synergism?

Arnold et al. 1996



E-Screen

MCF7 Human Breast Cancer Cell Culture



E-Screen Measurements

(PE)
Proliferative Effect = $\frac{\text{maximum cell yield for test chemical}}{\text{maximum cell yield control}}$

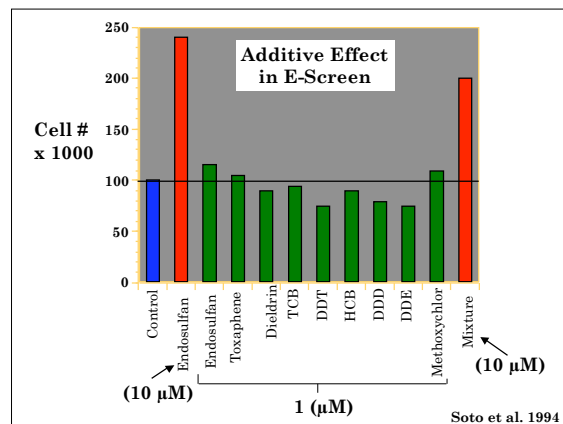
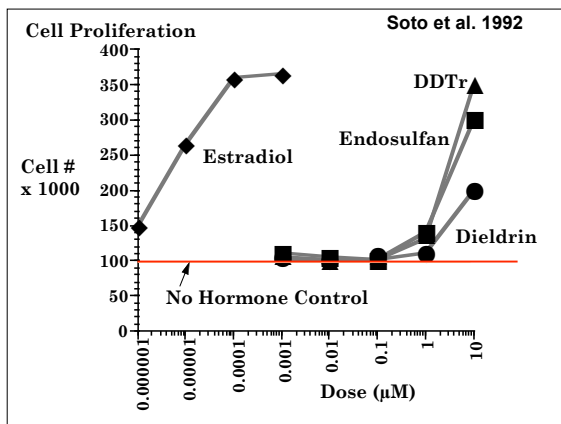
(RPE)
Relative Proliferative Effect = $100 \times \frac{\text{max. cell yield test chem.}}{\text{max. cell yield estradiol}}$

(RPP)
Relative Proliferative Potency = $\frac{\text{min. conc. E2 for max yield}}{\text{min. conc. test chem. max yield}}$

E-Screen Results

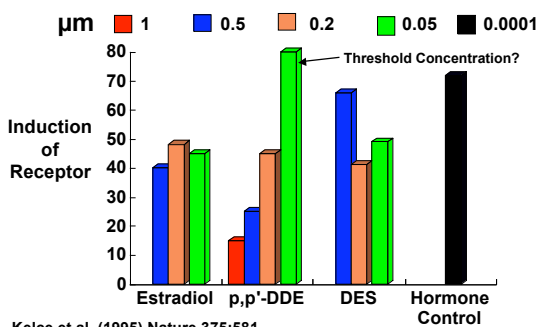
Chemical	Concen.	PE	RPE (%)	RPP (%)
estradiol	30 pM	6.7	100	100
DES	100 pM	5.1	112	1000
p,p'-DDT	30 μ M	5.0	70	0.0001
o,p'-DDT	30 μ M	5.8	84	0.0001
chlordane	30 μ M	1.3	5	-
zearalenone	3 nM	6.0	88	1

Soto et al. 1992



Soto et al. 1994

Anti-Androgenic Effects: Is There a Threshold?



Kelce et al. (1995) Nature 375:581

Competitive Binding Assays

Chemical	Androgen Receptor	Estrogen Receptor	5 α -reductase
estradiol	0.5	0.002	50
p,p'-DDT	75	>1000	>1000
p,p'-DDE	5	>1000	>1000
o,p'-DDT	95	5	>1000
Kepone	125	3	200
DES	10	0.0008	50

Kelce et al. 1995

In Vivo Assays for HAAs

- Uterine growth (if estrogenic)
- Prostate gland growth
- Nipples in male rats
- Feminization (morphological)
- Behavioral observations (work if behavior is gender specific)
- Vitellogenin biomarker in plasma or body

Note: Doses tend to be very high compared to levels of environmental exposure

Verdeal & Ryan 1979

Potency of Phytoestrogens

Phytoestrogen	Relative Potency (uterine growth)	Relative Affinity for estrogen receptor
DES	100000	
Estradiol		100
Estrone	6900	
Coumestrol	35	10
Zearalenone		3.3
Genistein	1	2
Daidzein	0.75	
Formononetin	0.26	0.01
Biochanin A	0.46	
o,p'-DDT		0.0001

In-Vivo Effects of DDE

- Pregnant rats dosed daily with 100 mg/kg bw DDE from day 14-18 of gestation
 - Males born with retained thoracic nipples (anti-androgenic effect)
- 25 day old rats dosed daily with 100 mg/kg bw DDE until day 57
 - Onset of puberty delayed by 5 days
 - No effect on whole body weight or testosterone levels in blood

Kelce et al. 1995

In-Vivo Effects of DDE

- Adult rats (120 days old) dosed daily with 100 mg/kg bw DDE for 4 days
 - Seminal vesicle wt. 84% of control
 - Ventral prostate wt. 70% of control
 - No effect on testosterone levels in blood (~6.6 ppb)

Kelce et al. 1995

Experimental Virtual Reality?

- Reported levels of DDT/DDE in blood of newborn rats fed 200 mg/kg bw/day averaged 2100 ppb (~6.6 μ M)
- One 1970 study of fetal blood reported 2.3 ppb (0.0073 μ M)

Reality Bites

- According to the FDA total diet monitoring study ('86 - '91), mean daily intake of DDT by a 6-11 month old human infant was 0.00005 mg/kg bw/day
- Thus, in the Kelce et al. (1995) study, pregnant rats on a daily basis were given ~2 million times the dose humans are normally exposed to

Dietary Intake of Pesticide EDCs

Daily Intake, 1986-1991

• o,p'-DDT	<0.0001 µg/kg/d
• p,p'-DDE	0.0441
• dicofol	0.0196
• dieldrin	0.0057
• endosulfan	0.0344
• methoxychlor	0.0004
• vinclozolin	0.0059

Gunderson et al. '95

Dietary Intake of Pesticide EDCs

Daily Intake, 1965-1967

• DDT	1.30 µg/kg/d
• Dieldrin	0.30 µg/kg/d

McGill et al. 1972

In-Vivo Dosing

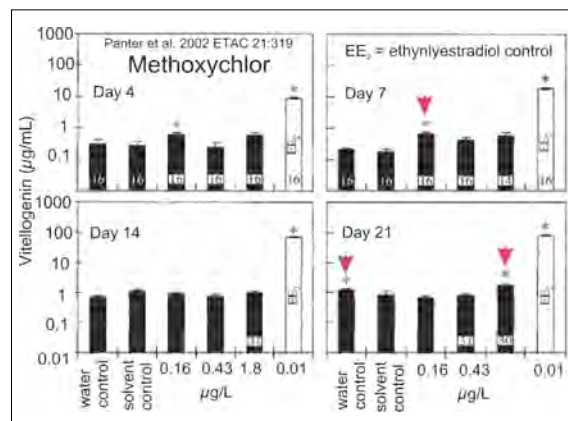
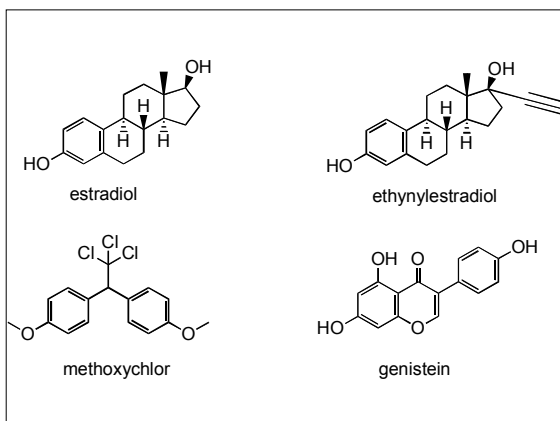
- Methoxychlor fed to weanling rats, daily through sacrifice or mating
 - 25, 50, 100, 200 mg/kg/d
- NOEL for sperm #, function, organ wts. = 25 mg/kg/d
- NOEL for vaginal opening & estrous = <25 mg/kg/d
- NOEL for fertility/offspring = 25 mg/kg/d

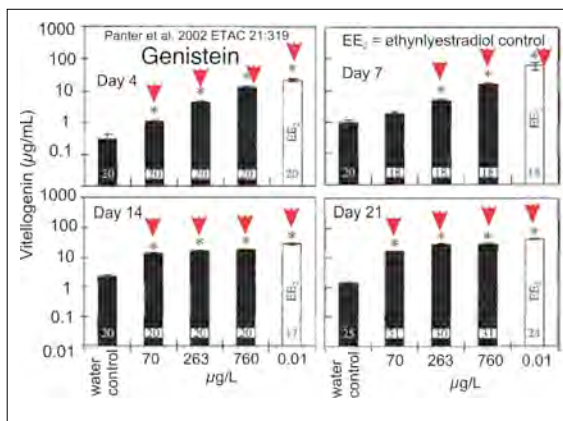
In-Vivo Testing

Juvenile Fathead Minnow Assay for Estrogenic or Anti-Estrogenic Effects

- Flow through study in aquaria
- Mixed sex juvenile fish exposed in water to nothing, synthetic estrogen (ethynylestradiol), or suspected HAAs
- Examine body weight, length, condition, and vitellogenin levels (in whole body) over 21 days

Panter et al. (2002) ETAC 21:319





In Vivo Testing: Screening chemicals for Juvenoid-Related Endocrine Activity Using Water Fleas

- *Daphnia magna* rely on methyl farnesoate (MF, a precursor to insect juvenile hormone I) for controlling development and for sex determination
- The presence of MF causes gravid female to produce males
- Expose gravid female to contaminants under conditions producing only females
- Measure males in the third brood

Wang et al. 2005 Aquatic Toxicol. 74:193

