Validation of EDSTAC Screening Tests: Uterotrophic Assay and 15-Day Intact Male Assay

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WIL Research Laboratories

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Uterotrophic Assay

- Background
- Scope of Validation
- Experimental Design
- Results – WIL Research
- Results – Comparison
Background

- Estrogen naturally regulates the growth of several female tissues: uterus, vagina, and mammary gland
- Estrogen stimulated growth of uterus is cyclic, rapid, and dramatic
- Rodent uterus presents opportunity for rapid assay for estrogen agonists and antagonists

Owens, 2002
History

- Assay developed in early 1930s
- Both rat and mouse models
- Used to detect estrogen mimics
Experimental Design

- Ovariectomized adult (42-56 days) or immature intact female rats (PND 17-20)
- Administer xenobiotic once per day for 3 consecutive days
- Euthanize 24 hours after last dose is administered
- Weigh freshly dissected (wet) and blotted uterus
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<tr>
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<td>Japan, JBRC</td>
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<td>Japan, CERI</td>
<td><strong>US, WIL Research Laboratories</strong></td>
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Test Substances

Ethinyl Estradiol

\( o,p'-\text{DDT} \)

Genistein

Bisphenol A

Methoxychlor

Nonylphenol

Dibutylphthalate
Validation Designs

**SUBCUTANEOUS (SC) ADMINISTRATION**
- Bypasses Intestinal Glucuronidation and Liver First-Pass Effect
- Vehicle Control
- 2 Levels EE
- 3 Dose Levels/Compound
- MC, G, BPA, DDT, NP
- 6 Animals/Group

**BLIND DOSING**
- Vehicle Control
- 2 Levels EE
- 1 Unknown Level Each: DBP, MC, G, BPA, DDT, NP
- 6 Animals/Group

**ORAL GAVAGE**
- More relevant for similarity to 2-Gen administration (dietary) and human exposures
- 18 Days at Randomization
- 30-50 g at Start
- Dose 19, 20, 21
- Euth. on 22

**INTACT IMMATURES**
- OVX at 6-7 Weeks
- 8-11 Weeks at Start
- >200 g at Start
- Dose 3 Days
- Euth. Day 4

**OVARIECTOMIZED ADULTS**
- Vehicle Control
- 2 Levels EE
- 1 Unknown Level Each: DBP, MC, G, BPA, DDT, NP
- 6 Animals/Group

2 Routes X 2 Models X 2 Designs = 8 Studies
Vehicle Control

Mean Uterine Weights - Vehicle Control (Corn Oil)

Route of Administration

SC
- Immature – WUW
- Immature – BUW
- Adult OVX – WUW
- Adult OVX – BUW

Oral
- Immature – WUW
- Immature – BUW
- Adult OVX – WUW
- Adult OVX – BUW

Grams

0.0000
0.0200
0.0400
0.0600
0.0800
0.1000
0.1200
0.1400

American Chemistry Council
Positive Control (Absolute)

Ovariectomized Model Mean Uterine Weights

![Bar chart showing wet and blotted uterine weights across different EE doses.](chart.png)
Positive Control (Relative)

Mean Uterine Weights - 17α-Ethynyl Estradiol (EE)

Dosage Levels (ug/kg/day) and Route

% of Control Mean

SC
- Immature – WUW
- Immature – BUW
- Adult OVX – WUW

Oral
- Immature – WUW
- Immature – BUW
- Adult OVX – WUW
- Adult OVX – BUW
## WIL Research Results

### Dose-Response Studies

<table>
<thead>
<tr>
<th>Test Article</th>
<th>Immature</th>
<th>Adult - OVX</th>
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<tr>
<td></td>
<td>Subcutaneous</td>
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<tr>
<td>EE (ug/kg/day)</td>
<td>0.3, 1.0</td>
<td>1.0, 3.0</td>
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<tr>
<td>MC (mg/kg/day)</td>
<td>100, 250, 500</td>
<td>50, 120, 300</td>
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<tr>
<td>G (mg/kg/day)</td>
<td>15, 35, 50</td>
<td>60, 120, 300</td>
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<tr>
<td>BPA (mg/kg/day)</td>
<td>100, 300, 600</td>
<td>200, 375, 600</td>
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<tr>
<td>DDT (mg/kg/day)</td>
<td>25, 50, 100</td>
<td>50, 125, 300</td>
</tr>
<tr>
<td>NP (mg/kg/day)</td>
<td>15, 35, 80</td>
<td>75, 125, 250</td>
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### Blinded-Design Studies

<table>
<thead>
<tr>
<th>Test Article</th>
<th>Immature</th>
<th>Adult - OVX</th>
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<tr>
<td></td>
<td>Subcutaneous</td>
<td>Oral</td>
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<tr>
<td>EE (ug/kg/day)</td>
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<td>0.075, 1.0</td>
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<tr>
<td>MC (mg/kg/day)</td>
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<td>G (mg/kg/day)</td>
<td>35</td>
<td>300</td>
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<tr>
<td>BPA (mg/kg/day)</td>
<td>300 (13+/1-)</td>
<td>600 (6+/4-)</td>
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<tr>
<td>DDT (mg/kg/day)</td>
<td>100</td>
<td>N/A</td>
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<tr>
<td>NP (mg/kg/day)</td>
<td>80</td>
<td>250</td>
</tr>
<tr>
<td>DBP (mg/kg/day)</td>
<td>500</td>
<td>1000</td>
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Green = Positive Response  
Red = Negative Response
Conclusions

• Both models (immature v. OVX) performed well and appeared to be qualitatively equivalent. There is no *a priori* reason to favor one animal model over the other.

• Oral administration elicited a detectable response by all weak agonists consistent with ADME considerations (e.g., liver first-pass metabolism and methoxychlor activation).

• Modest differences in chemical-specific responses between the models may be a result of route- and/or age-specific ADME.

• Dose levels selected for oral administration of 17α-Ethynyl Estradiol to the adult OVX model did not elicit a uterine response.
Conclusions

• When the identities of the coded chemicals were revealed, with the possible exception of bisphenol A, good concordance for each chemical was observed between the uterine responses from the blind, multichemical study and the uterine responses when the identical dose was employed in the corresponding dose-response study.

• The uterine response was negative for the negative control article (dibutylphthalate, DBP) in both models and by both routes of administration.
15-Day Intact Male Assay

- Principle of Test
- Experimental Design
- Results – WIL Research
Background

- A screening assay used to detect various endocrine-acting compounds
- Alternative to Hershberger & Pubertal Female Combination
- Developed by DuPont Haskell Laboratory
  - John O’Connor
  - Jon Cook
  - Michael Kaplan
  - Steven Frame
  - Leonard Davis
Mode of Action Detection

• Receptor Agonists and Antagonists
  – Estrogen Receptor
  – Androgen Receptor
  – Progesterone Receptor
  – Dopamine Receptor

• Steroid Biosynthesis Inhibitors
  – 5α-Reductase
  – Aromatase Biosynthesis
  – Testosterone Biosynthesis

• Compounds that Alter Thyroid Homeostasis
General Study Design

15 Intact Males/Group
BW & Dose
15 Days
Necropsy & OW following Last Dosing
Collect Blood for Hormone Analyses
Microscopic Examination of Selected Organs

Young Adults
Route Considerations

Spectrum of Effects
Hormonal Cascade Measurements

RIA = Radioimmunoassay
CLA = Chemiluminescence Assay

RIA
DHT
PRL
LH
FSH
T4
TSH

CLA
E2
T
T3
T4
TSH

RIA

Hypothalamus

GnRH

Dopamine

TRH

Pituitary

TSH

FSH

LH

PRL

5A-R

Thyroid

TSH

T3

T4

Testes

FSH

LH

PRL

5A-R

Prostate

DHT

T

Adipose

E2

Aromatase

RIA = Radioimmunoassay
CLA = Chemiluminescence Assay
Considerations for Male Hormone Assays

- Time of necropsy - want a narrow window
  - Time since last dose
  - Ultradian & circadian rhythms of hormone secretion or cyclicity of endogenous hormone levels (e.g., TSH, prolactin)
  - Method of euthanasia - LH, testosterone, prolactin affected by acute stress
Organ Weights

- Testes
- Epididymides
- Prostate
- Seminal Vesicles/Coagulating Glands/Fluid
  - Prostate/Seminal Vesicles/Coagulating Glands
- Thyroid/Parathyroid
- Liver

O’Connor, personal correspondence
Hormone Spectra

<table>
<thead>
<tr>
<th>Steroid Biosynthesis Inhibitor Agent</th>
<th>T</th>
<th>DHT</th>
<th>E2</th>
<th>PRL</th>
<th>LH</th>
<th>FSH</th>
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O’Connor, et al., 1998
## Hormone Spectra

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O’Connor, et al., 1999
## Hormone Spectra

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<th>PR</th>
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Details of Current Study Design

- 17α-Alkylated Androgen
- Gavage
- 0, 30, 100, 300 mg/kg/day/15 Days
- Euthanize within two hours of final dose administration
- Weigh Organs
- Evaluate Hormone Levels
- Histopathology
Body Weights

Study Day

Grams

0 mg/kg/day
10 mg/kg/day
30 mg/kg/day
100 mg/kg/day
300 mg/kg/day
Hormone Analyses

mg/kg/day

% of Control Mean

T, DHT, LH, FSH, E2, PRL, TSH, T3, T4
Organ Weights as % of Control Mean
Relative Organ Weights as % of Control Mean

% of Control Mean Relative Weight

Final Body Weight, Testes, Prostate, Pros/Sem Ves/C and Sem Ves/C and Fluid, Epididymides, Liver, Thyroids/Parathyroids

0 mg/kg/day, 10 mg/kg/day, 30 mg/kg/day, 100 mg/kg/day, 300 mg/kg/day
Advantages over Hershberger Assay

• Uses intact animals, not orchidoepidididyectomized

• Not limited to androgenicity detection

• Mode of action detection

• Match unknowns to endocrine “fingerprint”
  – O’Connor
15-Day Intact Male Assay

Key References


