Complex mixtures of anti-androgens at concentrations below individual chemical effect levels produces reproductive tract malformations in the male rat

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Disclosure/Disclaimer

No conflicts of interest to disclose

This presentation does not necessarily reflect the views or policy of USEPA
Declines in male reproductive health

Environmental exposure
Genetic defects and polymorphisms

Lifestyle factors
Epigenetic factors

Testicular dysgenesis

Decreased Leydig cell function
Disturbed Sertoli cell function

Decreased INSL3 production
Decreased testosterone production

Impaired germ cell differentiation

Hypospadias
Short AGD

Cryptorchidism
Decreased testosterone production
Impaired spermatogenesis

GCNIS Testicular cancer

Reduced male fecundity influencing pregnancy rates

Skakkebaeck et al. 2016
Exposures are mixtures

Examples of multiple residue detections in CDC-NHANES

Pumarega et al. 2016
2003/2004 Cohort
n = 4,793
OCs, PCBs, PBDEs, PCDDs/Fs, PFCs

Qian et al. 2015
2007/2008 Cohort
n = 2,604
DEHP, DiBP, DBP, DINP, BBP, DIDP
Chemical mixtures research and risk assessment in federal laws

Food Quality Protection Act

“(C) EXPOSURE OF INFANTS AND CHILDREN.—In establishing, modifying, leaving in effect, or revoking a tolerance or exemption for a pesticide chemical residue, the Administrator—
“(i) shall assess the risk of the pesticide chemical residue based on—
“(I) available information about consumption patterns among infants and children that are likely to result in disproportionately high consumption of foods containing or bearing such residue among infants and children in comparison to the general population;
“(II) available information concerning the special susceptibility of infants and children to the pesticide chemical residues, including neurological differences between infants and children and adults, and effects of in utero exposure to pesticide chemicals; and
“(III) available information concerning the cumulative effects on infants and children of such residues and other substances that have a common mechanism of toxicity; and
“(ii) shall—
“(I) ensure that there is a reasonable certainty that no harm will result to infants and children from aggregate exposure to the pesticide chemical residue; and

Safe Drinking Water Act

The Administrator shall conduct biomedical studies to—

(1) understand the mechanisms by which chemical contaminants are absorbed, distributed, metabolized, and eliminated from the human body, so as to develop more accurate physiologically based models of the phenomena;

(2) understand the effects of contaminants and the mechanisms by which the contaminants cause adverse effects (especially noncancer and infectious effects) and the variations in the effects among humans, especially subpopulations at greater risk of adverse effects, and between test animals and humans; and

(3) develop new approaches to the study of complex mixtures, such as mixtures found in drinking water, especially to determine the prospects for synergistic or antagonistic interactions that may affect the shape of the dose-response relationship of the individual chemicals and microbes, and to examine noncancer endpoints and infectious diseases, and susceptible individuals and subpopulations.
How do we group chemicals for cumulative risk assessment?
Androgen receptor antagonism

Reduced AR dependent mRNA/protein synthesis

Abnormal cell apoptosis/proliferation

Reduced androgen dependent tissue weights

Reduced sperm production

Suppressed development of gubernacular cords

Undescended testes

Reproductive tract malformations

Reproductive tract cancers

Infertility

Molecular Initiating Events

Key Events

Key Events

Adverse Outcomes

Adverse Outcomes
“Anti-androgen” AOP network

- 5α-reductase inhibition
- Androgen receptor antagonism
- Inhibition of CYP450 steroidogenic enzymes
- Inhibition of HMG-CoA reductase
- Unknown MIE

Molecular Initiating Events

Key Events

Adverse Outcomes

- Reduced DHT synthesis
- Reduced AR dependent mRNA/protein synthesis
- Reduced testosterone synthesis
- Reduced cholesterol synthesis
- Suppressed development of gubernacular cords
- Reduced sperm production
- Reduced androgen dependent tissue weights

Key Events

- Abnormal cell apoptosis/proliferation

Adverse Outcomes

- Reproductive tract malformations
- Reproductive tract cancers
- Undescended testes
- Infertility

- Reduced androgen dependent tissue weights
- Reduced sperm production
- Reduced DHT synthesis
- Reduced testosterone synthesis
- Abnormal cell apoptosis/proliferation

- 5α-reductase inhibition
- Androgen receptor antagonism
- Inhibition of CYP450 steroidogenic enzymes
- Inhibition of HMG-CoA reductase
- Unknown MIE
Hypothesis

Complex mixtures of many chemicals with multiple “anti-androgenic” molecular initiating events act additively at low doses to disrupt male reproductive tract development

“Low”: below individual chemical effect levels (LOAEL or NOAEL)
“Anti-androgen” AOP network

- **Finasteride**
  - 5α-reductase inhibition
  - Reduced DHT synthesis

- **p’p’-DDE**
  - Pyrifluquinazon
  - Procymidone
  - Vinclozolin
  - Flutamide
  - Androgen receptor antagonism
  - Reduced AR dependent mRNA/protein synthesis
  - Suppressed development of gubernacular cords
  - Undescended testes
  - Reproductive tract malformations
  - Reproductive tract cancers

- **Prochloraz**
  - Linuron
  - Inhibition of P450 steroidogenic enzymes
  - Reduced testosterone synthesis

- **Simvastatin**
  - Inhibition of HMG-CoA reductase
  - Reduced cholesterol synthesis

- **Phthalates**
  - Unknown MIE
  - Molecular Initiating Events

- **Key Events**
  - Abnormal cell apoptosis/proliferation
  - Reduced sperm production
  - Reduced androgen dependent tissue weights
  - Infertility
  - Adverse Outcomes
## Individual chemical effect levels

<table>
<thead>
<tr>
<th>Chemical</th>
<th>LOAEL (mg/kg/d)</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finasteride</td>
<td>0.03</td>
<td>AGD red., Nipple ret.</td>
<td>Clark 1993</td>
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<tr>
<td>Flutamide</td>
<td>2.0</td>
<td>Hypospadias</td>
<td>Welsh 2009</td>
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<tr>
<td>Vinclozolin</td>
<td>6.0</td>
<td>VP wt., AGD red., Nipple ret.</td>
<td>Gray 1999; Hellwig 2000</td>
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<td>Diethylhexyl phth.</td>
<td>11.0</td>
<td>Various malf.</td>
<td>Gray 2009; Blystone 2010</td>
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<tr>
<td>Linuron</td>
<td>12.5</td>
<td>Testis &amp; epididymal malf.</td>
<td>McIntyre 2000</td>
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<tr>
<td>Procymidone</td>
<td>25.0</td>
<td>AGD red., Nipple ret., Various malf.</td>
<td>Ostby 1999</td>
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<td>Pyrifluquinazon</td>
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<td>Various malf.</td>
<td>Unpublished</td>
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<tr>
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<td>31.3</td>
<td>Testis malf., T prod.</td>
<td>Blystone 2007; Noriega 2005</td>
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<td>Dipentyl phth.</td>
<td>33.0</td>
<td>T prod.</td>
<td>Hannas 2011; Furr 2014</td>
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<td>Simvastatin</td>
<td>62.5</td>
<td>Testis malf., T prod.</td>
<td>Beverly 2014</td>
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<td>p,p’-DDE</td>
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<td>AGD red., Nipple ret., PPS delay</td>
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<td>Diisooctyl phth.</td>
<td>227.0</td>
<td>Red. sperm count</td>
<td>McKee 2006</td>
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<td>AGD red.</td>
<td>Saillenfait 2009</td>
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<td>Benzylbutyl phth.</td>
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<td>Tyl 2004</td>
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<td>AGD red.</td>
<td>Saillenfait 2011</td>
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<td>1/5</td>
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<td>1/10</td>
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<tr>
<td>1/20</td>
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<td>1/40</td>
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<td></td>
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<tr>
<td>1/80</td>
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</tbody>
</table>
Postnatal reproductive development

- Charles River Sprague-Dawley
- Oral gavage administration
- 5 dams/litters per dose group
- Corn oil negative control

AGD = anogenital distance
NR = nipple retention
VO = vaginal opening
PPS = preputial separation
Early postnatal endpoints
Adult reproductive tissue weights

Ventral prostate wt (mg)

Glans penis wt (mg)

Paired testis wt (g)

Paired epididymis wt (mg)

Paired seminal vesicle wt (g)

LABC wt (g)

0
200
400
600
800
1000

0
2
4
6
8
10

0
0.5
1
1.5
2

0
0.5
1
1.5
2

p=0.063

p=0.061

p=0.064

p=0.061

p=0.060

p=0.052

p=0.055

p=0.051
Ultramix Summary

- Cumulative effects of many “anti-androgens”
- Effects appeared to conform to dose addition
- Adverse reproductive tract effects occurred low doses (LOAEL/80)
- Concerns:
  - No doses in ultra low dose range (non-monotonic effects)
  - Very high potency of drugs – finasteride/flutamide
- Additional questions:
  - Accuracy of mixture models (Dose Addition vs. Response Addition)
  - Benchmark Dose Modeling
# Supermix dosing

<table>
<thead>
<tr>
<th>Dose</th>
<th>[Chemical]</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% Dose</td>
<td>NOAELx2</td>
</tr>
<tr>
<td>50% Dose</td>
<td>NOAEL</td>
</tr>
<tr>
<td>25% Dose</td>
<td>NOAEL/2</td>
</tr>
<tr>
<td>12.5% Dose</td>
<td>NOAEL/4</td>
</tr>
<tr>
<td>6.25% Dose</td>
<td>NOAEL/8</td>
</tr>
<tr>
<td>3.3% Dose</td>
<td>NOAEL/15</td>
</tr>
<tr>
<td>0.5% Dose</td>
<td>NOAEL/100</td>
</tr>
<tr>
<td>0.05% Dose</td>
<td>NOAEL/1000</td>
</tr>
</tbody>
</table>
Early postnatal endpoints
Adult reproductive tissue weights

- Ventral prostate wt (mg)
- Glans penis wt (mg)
- Paired testis wt (g)
- Paired epididymus wt (mg)
- Paired seminal vesicle wt (g)
- LABC wt (g)

Graphs showing the weight distribution of various reproductive tissues under different conditions, with NOAEL values indicated for each condition.
Adult external malformations

% males with nipples

Hypoplasias (% of litter)

Noriega et al. (2005)
Adult internal malformations

Control

Supermix 50% and 100% doses (Chemicals at NOAEL and 2xNOAEL)

*Male sex accessory tissues absent in:
95% of males at 100% dose
54% of males at 50% dose
Mixture models of AGD

Super mix male observed
$ED_{50}: 32.6-44.2\%$ (95\% CI)

Dose addition model
$ED_{50}: 41.3\%$

Response addition model
$ED_{50}: 52.4\%$
Mixture models of Hypospadias

Super mix male observed
ED$_{50}$: 22.8 - 25.9% (95% CI)

Dose addition model
ED$_{50}$: 38.4%

Response addition model
ED$_{50}$: 624.8%
Benchmark Dose estimation

\[ BMD_{90} = 8.9 \% \text{top} \]
\[ BMDL_{90} = 6.2 \% \text{top} \approx \text{NOAEL/8} \]
Summary

- Chemicals with multiple “anti-androgenic” molecular initiating events act cumulatively to disrupt male reproductive tract development in the male rat.

- Effects occur when component chemicals are at low individual doses.
  - Significant reductions in tissue weights in Ultramix at LOAEL/80.
  - Significant reductions in fetal T production in Supermix at NOAEL/8.

- Utility of AOPs for grouping chemicals that affect similar biological pathway.
  - Grouping based on common MIE is too restrictive.
  - Overlap of critical KEs can identify chemicals for cumulative risk assessment.

- Dose addition model more accurate than Response addition model.

- Some of the most complex developmental studies on reproductive toxicants to date.
  - Very few researchers/laboratories are studying in utero exposure to mixtures.

- Exposure to environmental chemicals that disrupt androgen signaling may be contributing to adverse male reproductive human health effects at doses lower than previous estimated.
Research Team

Earl Gray
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Christy Lambright
Nicki Evans
Elizabeth Medlock-Kakaley
Hunter Sampson*
Johnathan Furr*

*no longer at USEPA
Questions?
Image References


