From Silent Spring to Silent Night

Dr. Tyrone Hayes
January 30, 2009
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Dr. Tyrone Hayes
University of California at Berkeley
January 30\textsuperscript{th}, 2009
ATRAZINE
ATRAZINE IS AN HERBICIDE USED WITH MONOCOT CROPS (CORN, SORGHUM) AND STONE FRUITS
ATRAZINE HAS BEEN USED FOR 48 YEARS
ATRAZINE

80 MILLION POUNDS USED ANNUALLY IN THE U.S.
ATRAZZINE IS USED IN MORE THAN 80 COUNTRIES
ATRAZINE

BUT NOW OUTLAWED IN ALL OF EUROPE
Laboratory Model
Testosterone
Aromatase

ATRAZINE

Estrogen
Blood testosterone (ng/mL)

Control Males
Atrazine-Treated Males
Control Females

Hayes et al. 2002
Hayes, unpubl. data

Control

Males
Females
Malformed

ATRAZINE (ppb)

Aromatase (CYP19)/EF-1-a E-04

A
B

Control

0.1

1.0

A
B

A
B

A
B

Hayes, unpubl. data
Successful Pairings

Hayes, unpubl. data
Percent Fertile

Control Males

Atrazine-Treated Males

Hayes, unpubl. data
1. Laboratory Model

2. Comparative Studies
ARE THE EFFECTIVE DOSES ($\geq 0.1$ PPB) ECOLOGICALLY RELEVANT?
Atrazine

No Atrazine

92% Hermaphrodites

0% Hermaphrodites
SPRING
Atrazine
Metolachlor
Alachlor
Nicosulfuron

Methoxyacetyl
Propiconazole
Tebupirimphos
Cyfluthrin
Cyhalothrin
Control

Atrazine and S-metolachlor

Mixture of 9 compounds

Hayes et al., 2006
0.6 ppb Atrazine

15.3 ppb Atrazine
Time to complete tail reabsorption (days)

Body Weight (mg)

Control

Atrazine + S-met

Mix
Stress hormone (Corticoids)

Immunosuppression, Decreased growth, Retarded development, Inhibited metamorphosis
Blood Cortisone (ng/mL)

<table>
<thead>
<tr>
<th></th>
<th>Control Males</th>
<th>Pesticide-treated Males</th>
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</thead>
<tbody>
<tr>
<td>Blood Cortisone</td>
<td></td>
<td></td>
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<tr>
<td>(ng/mL)</td>
<td>1</td>
<td>3.5</td>
</tr>
</tbody>
</table>

The graph shows a comparison of blood cortisone levels between control males and pesticide-treated males. The pesticide-treated males have significantly higher blood cortisone levels compared to the control group.
Stress hormone (Corticoids)
Atrazine increases rana virus susceptibility in the tiger salamander, *Ambystoma Tigrinum*
1. Laboratory Model
2. Comparative Studies
3. Field Studies
4. Field Simulations

Diagram flow: 1 -> 2 -> 3 -> 4 -> 1
Santa Margarita | Atascadera | Salinas

Length (mm)

- Santa Margarita: 120
- Atascadera: 60
- Salinas: 0
Yeast injection (g/ml)

Percent total

Santa Margarita

Atascadera

Salinas

Yeast injection (g/ml)
“In ecoepidemiology, the occurrence of an association in more than one species and species population is very strong evidence for causation.” (Fox, ref. 10, page 368).
“There is no direct scientific information to assess this hypothesis.”

from EPA, Statement of Anne Lindsay, page 10
Atrazine

Testosterone

Sperm production
In rats (Kniewald et al. 2000) $P < 0.001$
In humans
Swan et al. 2003

$P < 0.009$
Atrazine level (ppb)

Control Males  Sub-fertile Males  Field Workers (Average)

Lucas et al. 1993
Atrazine level (ppb)

<table>
<thead>
<tr>
<th></th>
<th>Control Males</th>
<th>Sub-fertile Males</th>
<th>Field Workers (Average)</th>
<th>Applicators (Maximum)</th>
</tr>
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<tbody>
<tr>
<td>Males</td>
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<tr>
<td>Sub-fertile</td>
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<td>Applicators</td>
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Lucas et al. 1993
“Atrazine has been a vital tool for US farmers for nearly 50 years.”

--Tim Pastoor, Letter to the editor
Terra Haute Tribune Star
Jan 3, 2008
Atrazine

Testosterone

\[ \text{aromatase} \]

\[ \text{Estrogen} \]

Sperm production

\[ \text{Vitellogenesis} \]

\[ \text{Oogenesis} \]

\[ \text{Mammary tumors} \]

\[ \text{Prostate cancer} \]
**Serum Testosterone (pg/ml)**

- **CONTROL (200 mg/kg)**
- **ATRAZINE**

**P < 0.05**

**Serum Estrogen (pg/ml)**

- **E2+E1**

**Atrazine (mg/kg)**

- **Stoker 2000 Rodents**
Mammary tumor incidence (%) for females treated with atrazine in rodents. A significant increase in incidence is observed at 70, 500, and 1000 ppm compared to the control group, with a p-value of <0.01.

Stevens et al. 1994

Rodents
Relative CYP 19 expression or aromatase activity

- Control
- Atrazine treated

$P < 0.05$

Sanderson et al. 2001

Human Cells
$P < 0.0001$

In humans
Kettles et al. 1997
“The increase in all cancers combined seen in the overall study group was concentrated in the company employee group.” (page 1052)

“The increase in prostate cancer in male subjects was concentrated in company employees.” (page 1052).

“The prostate cancer increase was further concentrated in actively working company employees.” (page 1053)

“all but one of the cases occurred in men with 10 or more years since hire”. (page 1052)

“Analyses restricted to company employees also found that the prostate cancer increase was limited to men under 60 years of age.” (page 1053).

MacLennan et al. 2002
Prostate cancer incidence (Observed/Expected)

Control Males
Exposed Males

MacLennan et al. 2002
“Atrazine has been used safely for more than 47 years.”

-- Tim Pastoor
Syngenta Crop Protection
“There is no direct scientific information to assess this hypothesis.”

(EPA, page 10)
Letrozole

Aromatase

Testosterone

Estrogen
Letrozole

\[ \text{Aromatase} \]

\[
\begin{align*}
\text{Testosterone} & \xrightarrow{\text{Aromatase}} \text{Estrogen} \\
\text{ATRAZINE} & \xrightarrow{\uparrow} \text{ER}
\end{align*}
\]
“Novartis Oncology offers treatments for cancers that range from breast cancer to leukemia to bone cancer to carcinoid tumors.” – novartis.com
Letrozole inhibits aromatase, preventing the conversion of testosterone to estrogen. ATRAZINE can also inhibit aromatase activity.
Aromatase

Testosterone

Estrogen

Letrozole

ATRAZINE
“...use of atrazine according to the label instructions will not likely result in harm to human health or the environment.”

-- Office of Pesticide Programs
US EPA, 2008
Working Hypothesis

1. Apical Effects
   - YES
   - NO: STOP

2. Sex Steroid Measurements
   - YES
   - NO: Test for Alternative Effects

3. Aromatase Activity?
   - YES
   - NO: Test for Alternative Effects

4. Aromatase Inhibitor Study
   - YES
   - NO

Ecological Relevance

Mechanism

<table>
<thead>
<tr>
<th></th>
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Working Hypothesis

1. Apical Effects
   YES
   2. Sex Steroid Measurements
      YES
      3. Aromatase Activity?
         YES
         4. Aromatase Inhibitor Study
            NO

Ecological Relevance

Mechanism

Test for Alternative Effects

STOP
Neural Damage
Immune Failure
Abortion
Impaired Mammary Development
Prostate Disease
Neural Damage
Prostate and Mammary Cancer
Abortion
Atrazine
Impaired Growth and Development

Neural Damage

Immune Failure

Abortion

Prostate Disease

Mammary Cancer

Prostate and Mammary Cancer
Impaired Growth and Development

Abortion

Impaired Mammary Development

Prostate Disease

Impaired Growth and Development
“The ultimate decision is much bigger than science…
…it weighs in public opinion.”

-- Stephen Bradbury
US EPA, Dec 3, 2006
Thank you

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Dr. Tyrone B. Hayes's work is transforming biology one battle at a time. From a very early age, he knew that he was interested in biology, amphibians in particular. Always fascinated by frogs and toads, this fascination turned into reality and a career. Dr. Hayes's work paves the way for an integrative approach to understanding how an organism reacts to changes in its environment.

Recently his work has gained international recognition for demonstrating how atrazine, a widely used pesticide which is now found in many water sources, has been shown to alter reproductive development (act as an endocrine disruptor) in frogs even at extremely low levels. Beyond his integrative work in conservation, he has won numerous teaching awards, conservation awards, and recognition as a leader in the scientific community.