Studying Biology to Understand Risk: Dosimetry Models and Quantitative Adverse Outcome Pathways

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RASS

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Disclaimer

This is a presentation of the opinions of Rory Conolly, not of official policies of the US EPA.
How do we get from here to there?
The short answer

- Understand the relevant biology
- Develop a computational model of the biology
- Simulate and make predictions of dose-response and time course
- Coordinate with decision-makers
The long answer: This presentation

- Biology as the underpinning to dose-response and risk
- Mechanistic studies and computational modeling to bridge from hazard to risk
- Quantitative AOP example
- Challenges in developing these models and using the predictions
What do we do?

Environmental fate & transport

Exposures

Hazards

Dose-responses & time-courses

Risks
What do we do?

Environmental fate & transport

Exposures

Hazards

Dose-responses & time-courses

Risks
Historically, the data haven’t answered the question.
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It’s the biology...
Parsing the problem: Pharmacokinetics & pharmacodynamics

Exposure → Tissue dose → Tissue interaction

Tissue interaction → Early tissue response → Irreversible pathology
Modern version:
Adverse Outcome Pathway

Exposure → Tissue dose → MIE

MIE → Key events → AO
Modern version:
Adverse Outcome Pathway

Exposure → Tissue dose → MIE

MIE → Key events → AO
Modern version:
Adverse Outcome Pathway

MIE \rightarrow \text{Key events} \rightarrow AO
Modern version: Adverse Outcome Pathway

Exposure → Tissue dose → MIE

Exposure → Tissue dose → MIE

MIE → Key events → AO

Exposure → Tissue dose → MIE
Historically, the data haven’t answered the question
The challenge

- How do we integrate laboratory data on ADME and AOPs to obtain quantitative understanding of dose-response and time-course behaviors?
The long answer: This presentation

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- Quantitative AOP example
- Challenges in developing these models and using the predictions
Computers are a big help!

(Intuitive modeling)

(Formal + Intuitive modeling)
The model reflects current understanding
“If I were a senior or first-year graduate student interested in biology, I would migrate as fast as I could into the field of computational biology.”

- Francis Collins, Director, NIH
A Physiologically Based Description of the Inhalation Pharmacokinetics of Styrene in Rats and Humans

JOHN C. RAMSEY* and MELVIN E. ANDERSEN†

*Toxicology Research Laboratory, Dow Chemical USA, Midland, Michigan 48640, and †Biochemical Toxicology Branch, Air Force Aerospace Medical Research Laboratory (AFAMRL/THB), Wright-Patterson Air Force Base, Ohio 45433
PBPK model
PBPK model

Important principles
- Relevant biology
- Parsimony

TOXICOLOGY AND APPLIED PHARMACOLOGY 73, 159–175 (1984)
(Styrene) in venous blood - 6 hr inhalation, 80 & 600 ppm
30-fold difference in blood concentration but 7.5-fold difference in inhaled concentration.
Formaldehyde dosimetry in the human nose
2082 pmol/(mm²-hr-ppm)

37 L/min

nasopharynx

Anterior nose

Whole nose
Power of PBPK - extrapolation
Power of PBPK - extrapolation
Power of biologically based modeling - extrapolation
Number of papers with "PBPK" or "Physiologically-Based Pharmacokinetic" in their title.  
(PubMed, Feb 2016)
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Modern version:
Adverse Outcome Pathway

In vivo

Exposure → Tissue dose → MIE

In vitro

MIE → Key events → AO
Aromatase inhibition leading to reproductive dysfunction in fish

Aromatase inhibition AOP
Small Fish Computational Toxicology Group

- **Academia**
  K. Watanabe, Oregan Health and Science University

- **USACE – Vicksburg, MS**
  M. Mayo, E. Perkins, N. Garcia-Reyero

- **USEPA (NHEERL) – Duluth, MN, and Grosse Ile, MI**

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  R. Conolly, W. Cheng (ISTD)
Molecular initiating event: Aromatase inhibition

Aromatase Inhibition → Granulosa Reduced E2 synthesis → Circulation Reduced E2 concentration → Hematocyte Reduced VIG production → Circulation Reduced VIG concentration → Ovary Impaired Oocyte Dev. → Female Decreased evolution/spawning → Population Declining Trajectory

Testosterone → Aromatase (CYP 19A) → 17β-estradiol (E2)
Modern version: Adverse Outcome Pathway

In vivo

Fadrozole → Tissue dose → MIE

In vitro

MIE → Key events → AO
Structure of the AOP

- Aromatase Inhibition
- Granulosa: Reduced E2 synthesis
- Circulation: Reduced E2 concentration
- Hepatocyte: Reduced VTG production
- Circulation: Reduced VTG concentration
- Ovary: Impaired Oocyte Dev.
- Female: Decreased ovulation/spawning
- Population: Declining Trajectory
Data supporting the AOP

Aromatase Inhibition → Granulosa Reduced E2 synthesis → Circulation Reduced E2 concentration → Hepatocyte Reduced VTG production → Circulation Reduced VTG concentration → Ovary Impaired Oocyte Dev. → Female Decreased ovulation/spawning → Population Declining Trajectory

Key events:
Reduced VTG in circulation

Aromatase Inhibition → Granulosa Reduced E2 synthesis → Circulation Reduced E2 concentration → Hepatocyte Reduced VTG production → Circulation Reduced VTG concentration → Ovary Impaired Oocyte Dev. → Female Decreased ovulation/spawning → Population Declining Trajectory

Plasma VTG (fold-change relative to control; log2)

- Control
- Fad 3 ug/L
- Fad 30 ug/L

Key events:
Impaired oocyte development & spawning

Aromatase Inhibition → Granulosa Reduced E2 synthesis → Circulation Reduced E2 concentration → Hepatocyte Reduced VTG production → Circulation Reduced VTG concentration → Ovary Impaired Oocyte Dev. → Female Decreased ovulation/spawning → Population Declining Trajectory

Toxi Sci 2002 67:121-130
Temporal concordance of key events

Aromatase Inhibition → Granulosa Reduced E2 synthesis → Circulation Reduced E2 concentration → Hepatocyte Reduced VTG production → Circulation Reduced VTG concentration → Ovary Impaired Oocyte Dev. → Female Decreased ovulation/spawning → Population Declining Trajectory

Ex vivo E2 (ng/ml)

6 Hour 12 Hour 24 Hour

A.

Plasma E2 (ng/ml)

6 Hour 12 Hour 24 Hour

B.

Plasma VTG (mg/ml)

6 Hour 12 Hour 24 Hour

C.

2011 Aquatic Toxicol. 103:170-178
Development of the QAOP
AOP $\Rightarrow$ QAOP

**Qualitative:** Defines association between a molecular initiating event and an adverse outcome.

**Quantitative:** Dose-response and time-course predictions
The QAOP: A combination of linked quantitative models

**HPG axis model**
- Aromatase Inhibition
- Granulosa Reduced E2 synthesis
- Circulation Reduced E2 concentration
- Hepatocyte Reduced VTG production
- Circulation Reduced VTG concentration

**Oocyte growth dynamics model**
- Ovary Impaired Oocyte Dev.
- Female Decreased ovulation/spawning

**Population dynamics model**
- Population Declining Trajectory
Fathead minnow HPG axis model
Homeostasis: Adaptation/Compensation

Exposure

Uptake-Delivery to Target Tissues

Perturbation

Cellular response pathway

Biologic inputs

“Normal” Biological Function

Early cellular changes

Adaptive Responses

Cell injury, Inability to regulate

Adverse Outcomes (e.g., mortality, Reproductive Impairment)
Adaptation: Plasma estradiol in fathead minnows exposed to fadrazole
HPG axis model:
Effect of aromatase inhibition on venous estradiol

A. Simulated Control
   - Measured Control

B. Simulated FAD 0.5 µg/L
   - Measured FAD 0.5 µg/L

C. Simulate FAD 3 µg/L
   - Measured FAD 3 µg/L

D. Simulate FAD 30 µg/L
   - Measured FAD 30 µg/L

Venous E2 (µM) vs. Time (day)
HPG axis model: Effect of aromatase inhibition on venous estradiol
HPG axis model:
Effect of aromatase inhibition on venous VTG

A. Simulated control
- Lab control

B. Simulated FAD 0.5 µg/L
- Lab FAD 0.5 µg/L

C. Simulated FAD 3 µg/L
- Lab FAD 3 µg/L

D. Simulated FAD 30 µg/L
- Lab FAD 30 µg/L
Oocyte growth dynamics model
(Egg development in the fathead minnow ovary)
Oocyte growth dynamics model: Predicts fecundity based on VTG levels

Prediction of normal fecundity vs Lab (mean) results at 21-days

Effects of fadrozole on predicted fecundity vs lab results
Population dynamics model:
Prediction of population dynamics

![Graph showing population dynamics](image)
The aromatase inhibition QAOP

**HPG axis model**
- **Aromatase Inhibition** → **Granulosa Reduced E2 synthesis** → **Circulation Reduced E2 concentration** → **Hepatocyte Reduced VTG production** → **Circulation Reduced VTG concentration**

**Oocyte growth dynamics model**
- **Ovary Impaired Oocyte Dev.** → **Female Decreased ovulation/spawning**

**Population dynamics model**
- **Population Declining Trajectory**
Does the new AOP terminology help?

- AOP specifies information needed to support regulatory decision making
  - Molecular initiating event
  - Key events
  - AO for individuals
  - AO for the population
- Richer language facilitates communication
The long answer: This presentation

- Biology as the underpinning to dose-response and risk
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- Quantitative AOP example
- Challenges in developing these models and using the predictions
Experimental design

* PBPK, BBDR, and qAOP models can simulate behavior of PK and the AOP over time
* So best supported by experimental designs that include both time-course and dose-response
Adaptation: Plasma estradiol in fathead minnows exposed to fadrazole
Formaldehyde

Dose-time response surface for regenerative cellular proliferation in nasal epithelium of the F344 rat.
Confidence (uncertainty$^{-1}$)

- **Concern:** The model increases uncertainty relative to not having the model.
  - Complicated structure relative to defaults
  - Errors in the model
  - Uncertainty about mechanism depicted in the model
Complicated structure...

- Delineation of sources of uncertainty does not mean uncertainty is increased.
- As long as good modeling practice is observed then model development coordinated with laboratory experiments is informative about roles of PK and key events.
- Uncovers hidden assumptions
Errors in model

- Coding errors definitely possible.
- But observation of good modeling practice, including rigorous code checking, addresses this concern.
Plenty of published guidance on good modeling practice

Physiologically Based Pharmacokinetic Model Use in Risk Assessment—Why Being Published Is Not Enough


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Uncertainty about mechanism

- This can be a valid concern but it applies to any work involving mechanisms, not just development of computational models.
- Addressed by peer review, scientific rigor
- Bradford Hill criteria
Return on the investment

• A fully developed QAOP is a powerful predictive tool.
  – Input exposure scenario of interest
  – Output prediction of change in adverse outcome

• But data needs are large
  – Expensive and time consuming
Return on the investment

Mature QAOP could serve as an “in silico” description of *in vivo* biology to aid in design of *in vitro* tests and interpretation of *in vitro* data.

HTS assays for MIE activation

*HPG axis model*

- Aromatase Inhibition
- Granulosa Reduced E2 synthesis
- Circulation Reduced E2 concentration
- Hepatocyte Reduced VTG production
- Circulation Reduced VTG concentration

*Oocyte growth dynamics model*

- Ovary Impaired Oocyte Dev.
- Female Decreased ovulation/spawning

*Population dynamics model*

- Population Declining Trajectory

*in vitro*  
*in vivo*
TEQ application to predict the population effects of mixture exposure

- Multi-component mixture
- 25 uM Fad and 25 uM Ima
- 26.5 uM TEQ fadrozole
Perfection

- Wanting perfection is a trap.
- The model should reduce uncertainty relative to where you stand without the model.
  - Model is only required to be useful.
- Sophisticated evaluation requires sufficient expertise in relevant biology, modeling technology, and an ability to “step back” and visualize the big picture.
"And that's why we need a computer."
If you don't know where you're going, you might not get there.
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  - CIIT / The Hamner (rip), 1989 - 2005
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    - Small fish group at MED
    - WanYun Cheng
End
Uncertainty

(unknown) upper bound on possible risk

Range of uncertainty where actual risk may exceed predicted risk

------ Best estimate of risk  ------

Range of uncertainty where actual risk may be lower than predicted risk

(unknown) lower bound on possible risk
How does uncertainty change as more data are incorporated?
Alternatively...

![Graph showing risk and information with upper and lower bounds and range of uncertainty.](image-url)
Virtual tissues: Dose at the cellular level
Dose-response predictions for the mixture of 8 chemicals after 21 days of exposure