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INNOVATIVE CELL BASED SCIENCE

45 Years Modeling Dose-Response Relationships: An Unanticipated Career!

Melvin E. Andersen
Distinguished Research Fellow
ScitoVation (www.scitovation.com)
New Orleans, LA
March 13, 2016

THE HAMNER INSTITUTES FOR HEALTH SCIENCES
WHERE GREAT MINDS & MEDICINE MEET

Part 1: Modeling measures of dose

- On becoming a **chemist** – or recognizing myself as one
- Getting derailed
- Moving into PK and **PBPK modeling** in the Navy and working for the Air Force
- **Finding a wide variety of applications for the PBPK tools**

- Providing instruction in PBPK modeling so others can get started more easily than I did

Opportunities

Resources & Technology

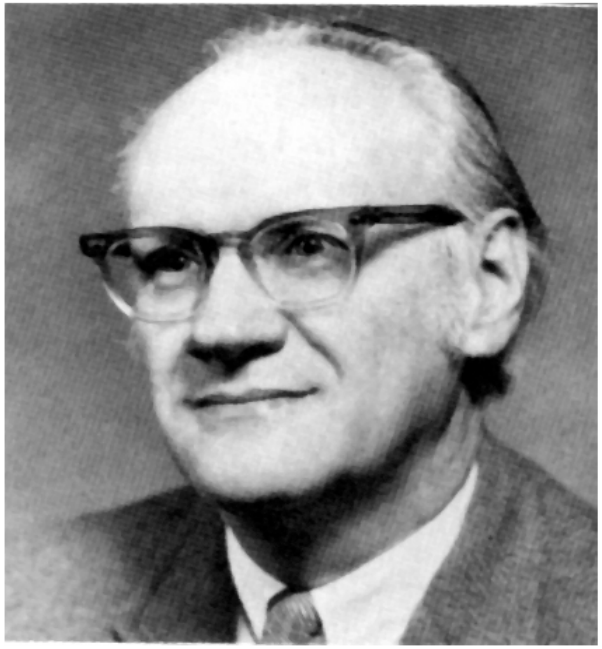
Staff, colleagues, supervisors, family, friends

Some, more than a small degree of good fortune

Going back to the beginning of my chemistry education

Latin–educo, educare–to lead out of

Chemistry–that which was led out from me



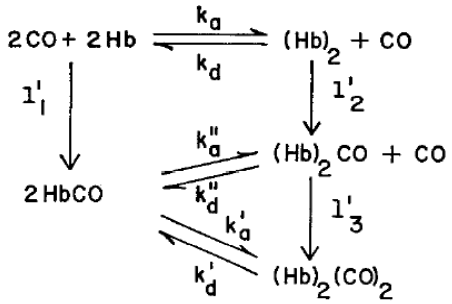
John O. Edwards, PhD
Department of Chemistry
Brown University

Quentin H. Gibson, MD, PhD
Department of Biochemistry & Molecular Biology
Cornell University

Andersen et al. (1969). The **Kinetics** of the Oxidation of Vanadyl Ion by Peroxodiphosphate in Aqueous Acid Solution. Inorgan. Chim. Acta 3, 655-659.

Andersen et al. (1971). **A Kinetic Analysis** of the Binding of Oxygen and Carbon Monoxide to Lamprey Hemoglobin: Petromyzon marinus and Petromyzon fluviatilis. J. Biol. Chem. 246, 4790-4799.

Ligand binding to Lamprey Hemoglobin



SCHEME 1

$$Hb + O_2 \underset{k_1}{\overset{k'_1}{\rightleftharpoons}} Hb\ O_2 \quad ; K_1 = \frac{(Hb)(O_2)}{(HbO_2)} = \frac{k_1}{k'_1}, \qquad (2)$$

$$Hb_2 + O_2 \underset{k_3}{\overset{k'_3}{\rightleftharpoons}} Hb_2O_2 \quad ; K_3 = \frac{(Hb_2)(O_2)}{(Hb_2O_2)} = \frac{k_3}{k'_3}, \qquad (3)$$

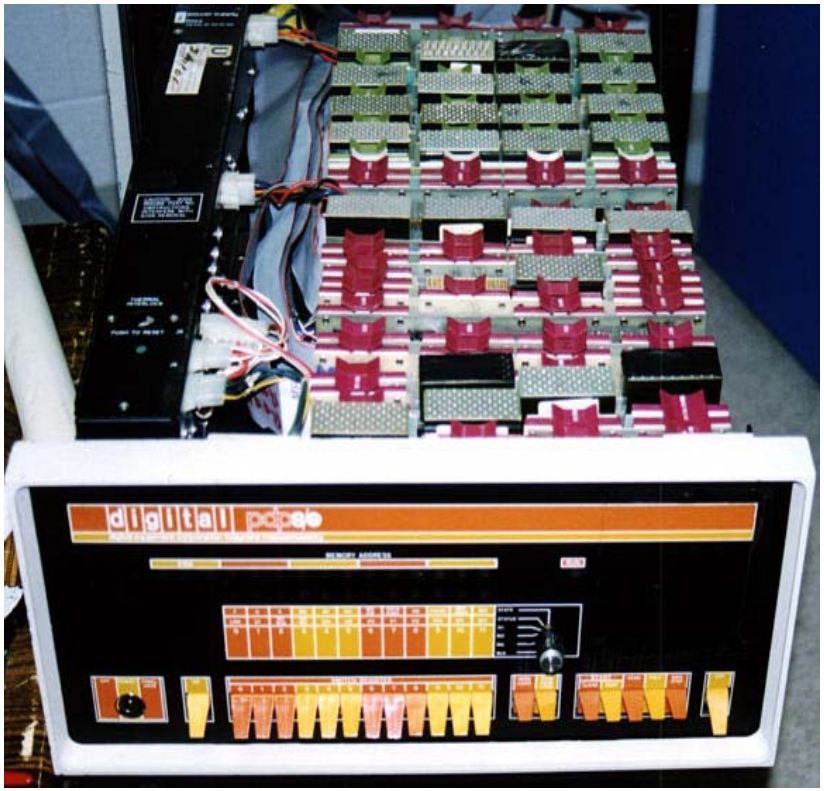
$$Hb_2O_2 + O_2 \underset{k_4}{\overset{k'_4}{\rightleftharpoons}} Hb_2(O_2)_2 \; ; K_4 = \frac{(Hb_2O_2)(O_2)}{(Hb_2(O_2)_2)} = \frac{k_4}{k'_4}, \qquad (4)$$

$$2HbO_2 \underset{k'_d}{\overset{k'_a}{\rightleftharpoons}} Hb_2(O_2)_2 \; ; K_5 = \frac{(HbO_2)^2}{(Hb_2(O_2)_2)} = \frac{k'_d}{k'_a}, \qquad (5)$$

$$HbO_2 + Hb \underset{k''_d}{\overset{k''_a}{\rightleftharpoons}} Hb_2O_2 \quad ; K_6 = \frac{(Hb)(HbO_2)}{(Hb_2O_2)} = \frac{k''_d}{k''_a}, \qquad (6)$$

SCHEME 2

My first experience modeling these differential equations used a PDP DEC 8I computer with **8K** memory - 1970



Duty called and instead of a postdoctoral at Woods Hole, I was off to Bethesda, MD (1971-1976) as a Navy Officer

Chemicals in Naval Environments – Propellants, CO2 Scrubbers, Atmospheric Contaminants

US Navy Toxicology Unit – Bethesda MD
Captain Jacob Siegel USN

Naval Medical Research Institute
Seymour Friess, PhD

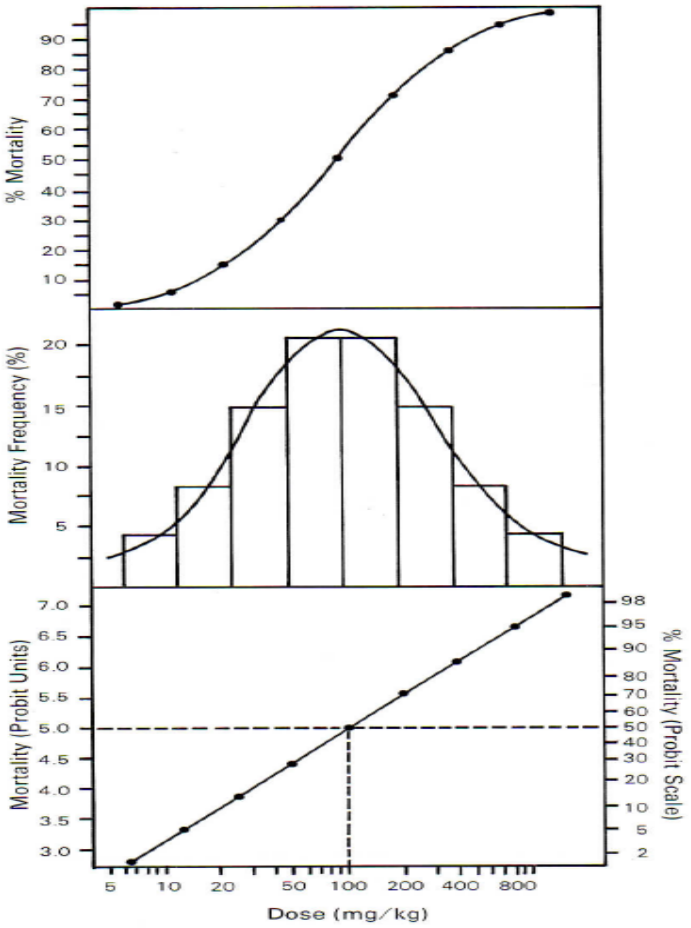
President: Seymour L. Friess

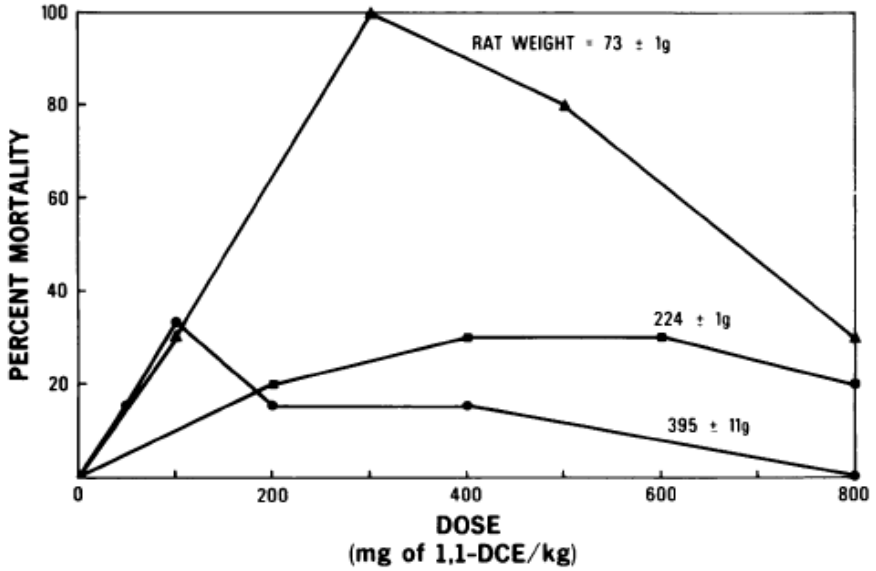
ON THE MECHANISM OF THE OXIDATION OF HUMAN AND RAT HEMOGLOBIN BY PROPYLENE GLYCOL DINITRATE*

MELVIN E. ANDERSEN and RICHARD A. SMITH
U.S. Navy Toxicology Unit, National Naval Medical Center, Bethesda, Md. 20014, U.S.A.

You can take the boy out of kinetics,
but not the kinetics out of the boy
(Larry Jenkins, Jr)

Step 1: Learn about and apply toxicological principles

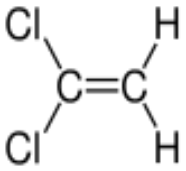




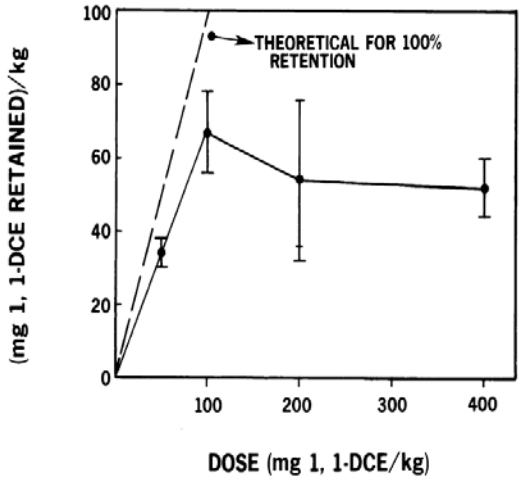
And, the data with vinylidene chloride sure didn't follow these expectations

I didn't quite understand why we used probit plots – is the world really log-normally distributed

On to Wright-Patterson, AFB, Ohio, still in the Navy



vinylidene chloride



Michael Gargas-1977

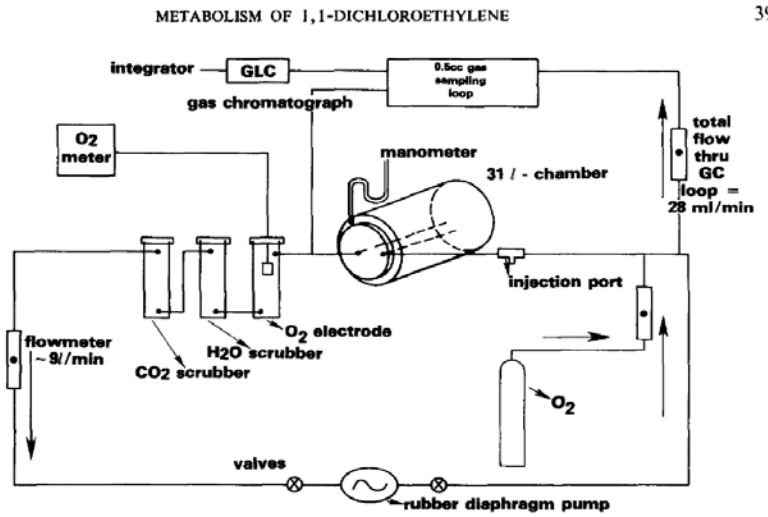
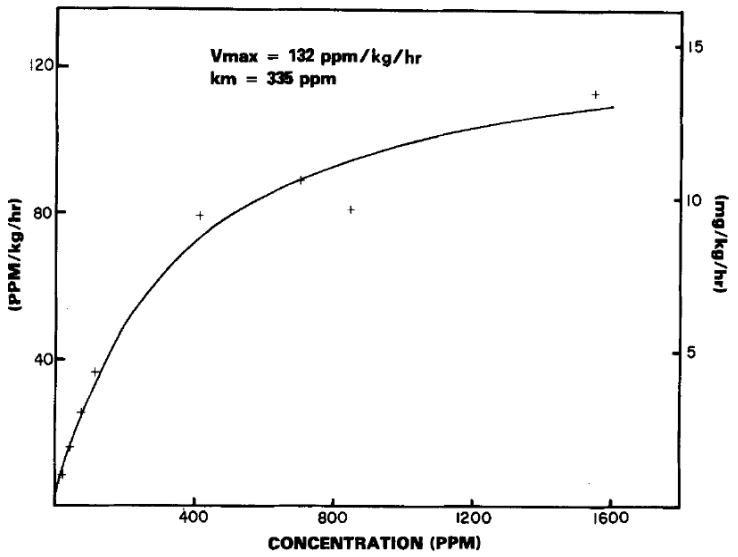


FIG. 1. Chamber configuration used for gas uptake studies.



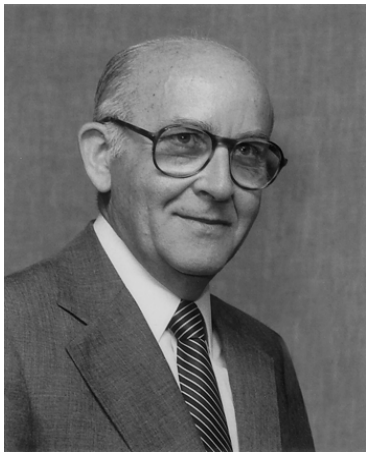
Gas Uptake System

At the end of 1978: Left the US Navy Toxicology Detachment moving down the hill to the Toxic Hazards Division, Armstrong Medical Research Laboratory, Wright Paterson AFB, OH

The Toxicology Branch Chief–**Ken Back**–and a Branch-PI, Marilyn George, were developing a program in PK modeling much like that pursued at Dow Chemical by Perry Gehring and his team.

SATURABLE METABOLISM AND ITS RELATIONSHIP TO TOXICITY*

Author: **Melvin E. Andersen**
Toxicology Branch
Air Force Aerospace Medical Research Laboratory
Wright-Patterson Air Force Base, Ohio



From the section

F. Interspecies Extrapolation of Toxicity Results:

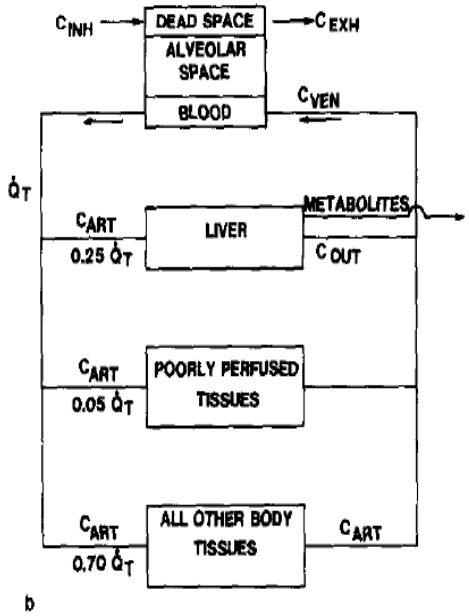
Leon Goldberg

This suggested approach to interspecies extrapolation of toxicity data relies on recent advances in modeling the pharmacokinetic behavior of therapeutic chemicals in various species. **The two techniques involved are development of realistic, physiologically based descriptions of pharmacokinetics in the test species [148-150] and application of scale-up techniques [151-152] to predict behavior based on the changing physiology between species.**

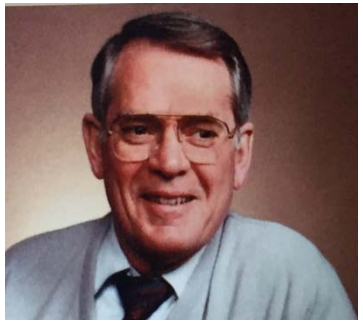
Career –changing activity

The beginning of PBPK at a New Orleans SOT Meeting-1979

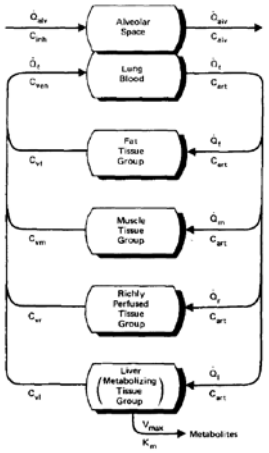
A Physiologically Based Toxicokinetic Description of the Metabolism of Inhaled Gases and Vapors: Analysis at Steady State[1]



Andersen, 1981



John Ramsey-1979



A Physiologically Based Description of the Inhalation Pharmacokinetics of Styrene in Rats and Humans[1]

JOHN C. RAMSEY*,[2] 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

Understand what is expected from a set of equations – especially the influence of clearance on the blood-air concentration ratio, but we needed better computational tools

Extrapolations across:

- concentrations
- dose routes
- species

Solved the equations on a Dow Mainframe

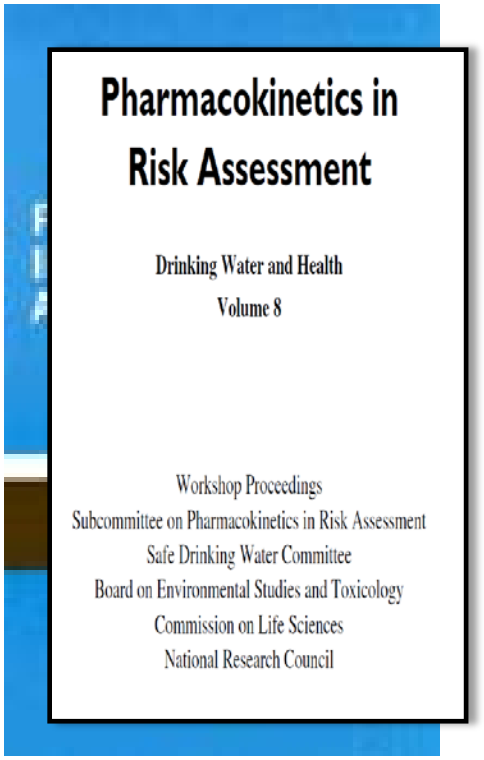
Harvey Clewell-1982

Toxicology and Industrial Health, Vol. 1, No. 4, 1985

RISK ASSESSMENT EXTRAPOLATIONS AND PHYSIOLOGICAL MODELING

HARVEY J. CLEWELL, III AND MELVIN E. ANDERSEN
Toxic Hazards Division
Air Force Aerospace Medical Research Laboratory
Wright-Patterson Air Force Base, OH 45433

Dick Reitz-1985

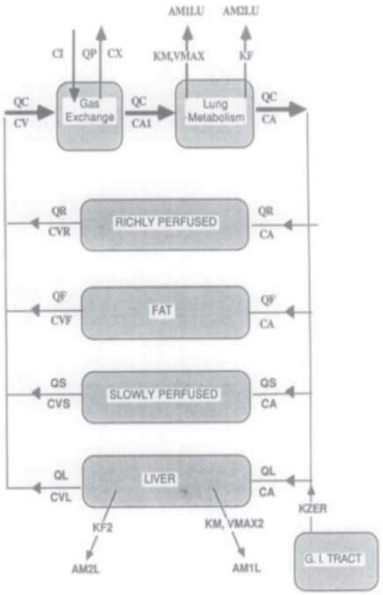


TOXICOLOGY AND APPLIED PHARMACOLOGY 87, 185–205 (1987)

Physiologically Based Pharmacokinetics and the Risk Assessment Process for Methylene Chloride

M. E. ANDERSEN, H. J. CLEWELL III, M. L. GARGAS, F. A. SMITH,* AND R. H. REITZ*

*Biochemical Toxicology Branch, Armstrong Aerospace Medical Research Laboratories, Wright–Patterson Air Force Base, Ohio 45433 and *Toxicology Research Laboratory, The Dow Chemical Company, U.S.A., 1803 Building, Midland, Michigan 48674*



A “good housekeeping” stamp of approval from the National Academy of Sciences–

PBPK is ready for prime time

WP-AFB staff putting PBPK models to work:

- Dermal uptake–**Jim McDougal**
- Cholinesterase inhibition & organophosphates–**Gary Jepson, Jeff Gerhardt**
- PBPK methods (gas uptake) and partition coefficient assays applied to large numbers of compounds– **Mike Gargas**
- Pregnancy and lactation exposures–**Jeff Fisher**
- In vitro constants as model input–**Dick Reitz with Fred Guengerich**
- Inhibitory interactions in vivo–**Kris Severyn**
- Moving on to non-volatile compounds–**Hon-Wing Leung and Dennis Paustenbach**

WP-AFB staff putting PBPK models to work:

- Metabolite formation–CO and bromide–**Mike Gargas**
- Glutathione depletion–**Richard D'Souza**
- Suicide inhibition of metabolism–**Mike Gargas, Harvey Clewell**
- Metabolic interactions of substrates and metabolites - hexane–all of us with data from **Doug Rickert**
- Risk assessment–sensitivity and variability analysis, parameter estimation, leading to reverse dosimetry and reverse causality in recent years–**Harvey Clewell**

- Developing PBPK/courses tutorials–**Dayton team**
- Providing 1 –2 week courses–**CSU starting in 1992**

Dermatotoxicity Conference New York City 1982

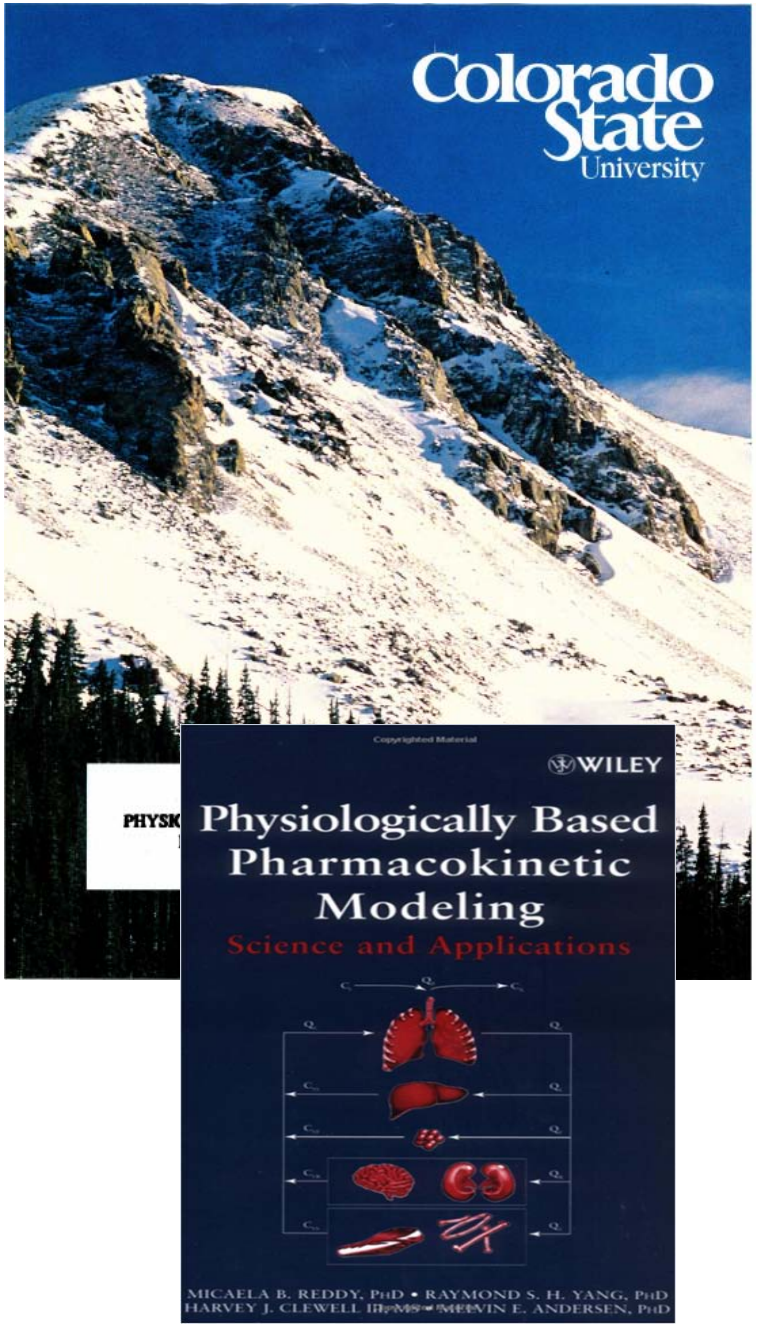
Toxicokinetic Principles in Relation to Percutaneous Absorption and Cutaneous Toxicity. The Fifth Conference on Cutaneous Toxicity, New York, NY, 16-18 May 1982.

Talked with Ray on the bus to the airport.

Asked about his paper just reviewed at TAP

“I like and honest man!”

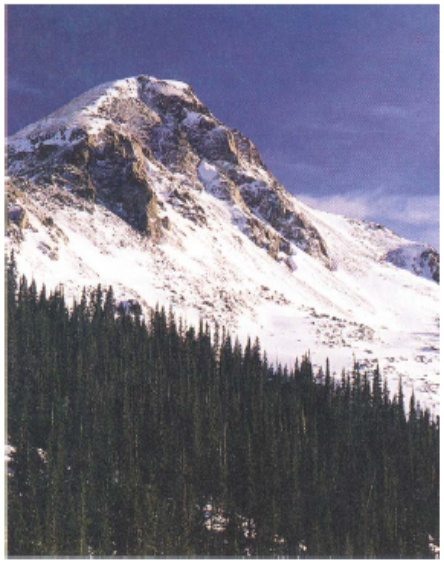
Raymond Yang



- Ray developed programs and research in mixture toxicology with an emphasis on PBPK and PBPD modeling.
- He established a biannual course in PBPK modeling in toxicology and risk assessment.
- By 1994, Ray, Harvey and I were the primary lecturers in the course.
- I joined Ray on the CSU faculty from 1999-2002.
- With students and postdoctoral staff, we completed a monograph on progress in PBPK modeling for a wide variety of compounds. **Michaela Reddy** took the lead in this effort.

Though retired, Ray is still doing a course in the summer, but also enjoying himself

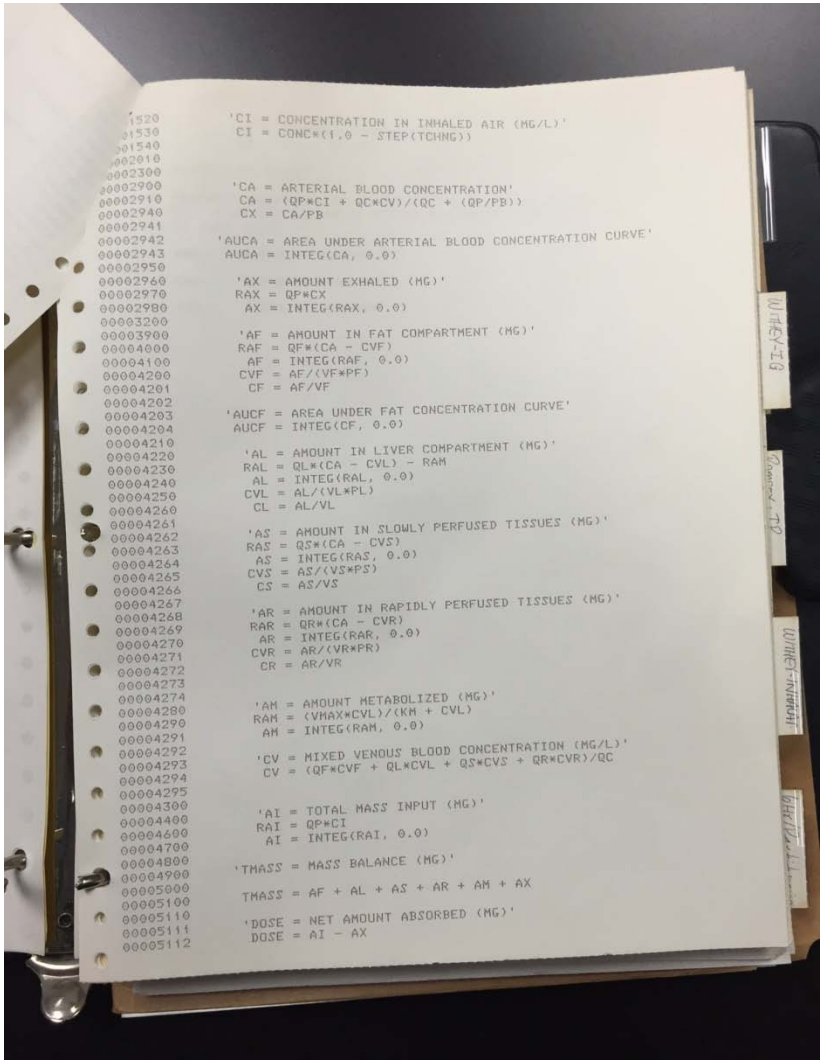
Physiologically-Based Pharmacokinetic Modeling Workshop for Beginners
Room 120, Environmental Health (EH) Bldg., Colorado State University, Fort Collins, Colorado, 01 - 05 August 2016



Description:

My emphasis is to teach physiologically-based pharmacokinetic (PBPK) modeling at a very basic level. The ultimate goal is that, at the end of the Workshop, participants can do their own PBPK modeling work using their own software (acquired for this Workshop as detailed below) and computers. For those of you whose primary interest is to understand application of PBPK modeling to risk assessment rather than to become PBPK modelers, my line-by-line detailed explanation and instruction of the code, as well as hands-on exercises, will enhance tremendously your appreciation of PBPK modeling and your risk assessment capability. Most of

Speeding the wider usage of PBPK tools

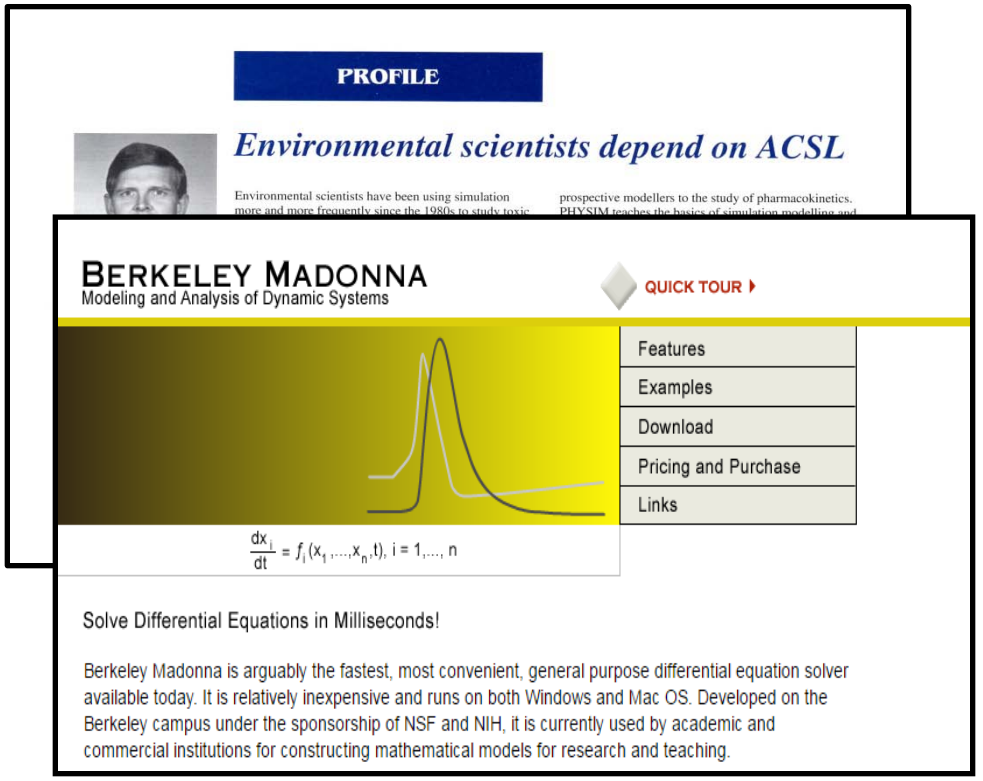


```
      'CI = CONCENTRATION IN INHALED AIR (MG/L)'
      CI = CONC*(1.0 - STEP(TCHNG))


    'CA = ARTERIAL BLOOD CONCENTRATION'
    CA = (QP*CI + QC*CV)/(QC + (QP/PB))
    CX = CA/PB

  'AUCA = AREA UNDER ARTERIAL BLOOD CONCENTRATION CURVE'
  AUCA = INTEG(CA, 0.0)

    'AX = AMOUNT EXHALED (MG)'
   RAX = QP*CX
    AX = INTEG(RAX, 0.0)

    'AF = AMOUNT IN FAT COMPARTMENT (MG)'
   RAF = QF*(CA - CVF)
    AF = INTEG(RAF, 0.0)
   CVF = AF/(VF*PF)
    CF = AF/VF

  'AUCF = AREA UNDER FAT CONCENTRATION CURVE'
  AUCF = INTEG(CF, 0.0)

    'AL = AMOUNT IN LIVER COMPARTMENT (MG)'
   RAL = QL*(CA - CVL) - RAM
    AL = INTEG(RAL, 0.0)
   CVL = AL/(VL*PL)
    CL = AL/VL

    'AS = AMOUNT IN SLOWLY PERFUSED TISSUES (MG)'
   RAS = QS*(CA - CVS)
    AS = INTEG(RAS, 0.0)
   CVS = AS/(VS*PS)
    CS = AS/VS

    'AR = AMOUNT IN RAPIDLY PERFUSED TISSUES (MG)'
   RAR = QR*(CA - CVR)
    AR = INTEG(RAR, 0.0)
   CVR = AR/(VR*PR)
    CR = AR/VR


    'AM = AMOUNT METABOLIZED (MG)'
   RAM = (VMAX*CVL)/(KM + CVL)
    AM = INTEG(RAM, 0.0)

    'CV = MIXED VENOUS BLOOD CONCENTRATION (MG/L)'
    CV = (QF*CVF + QL*CVL + QS*CVS + QR*CVR)/QC


    'AI = TOTAL MASS INPUT (MG)'
   RAI = QP*CI
    AI = INTEG(RAI, 0.0)

 'TMASS = MASS BALANCE (MG)'

 TMASS = AF + AL + AS + AR + AM + AX

  'DOSE = NET AMOUNT ABSORBED (MG)'
  DOSE = AI - AX
```

PBPK Historical Artifact # 1

Standard modeling code–Ramsey-an code

And, readily accessible programming languages, first ACSL and then Berkeley Madonna



PROFILE

Environmental scientists depend on ACSL

Environmental scientists have been using simulation more and more frequently since the 1980s to study toxic

prospective modellers to the study of pharmacokinetics. PHYSIM teaches the basics of simulation modelling and

BERKELEY MADONNA

Modeling and Analysis of Dynamic Systems

QUICK TOUR

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Examples
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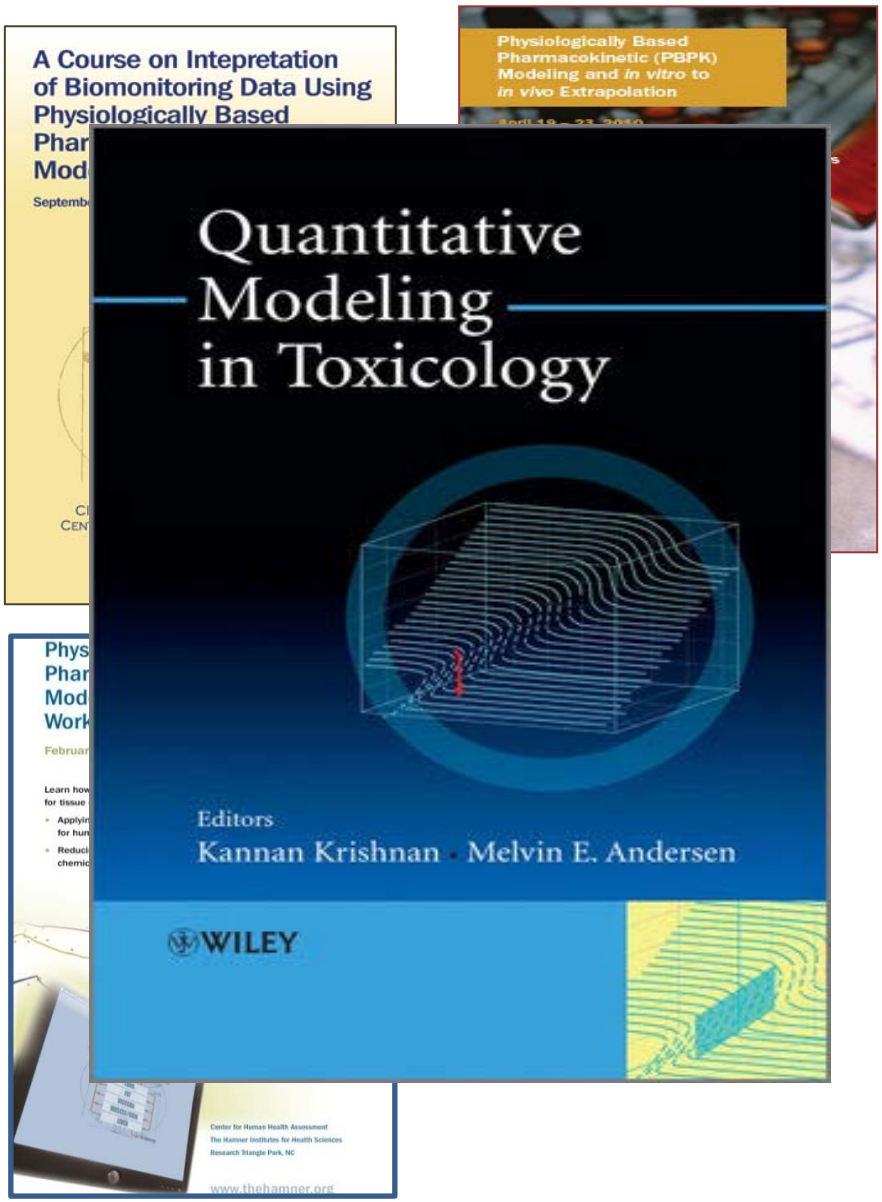
Links

$$\frac{dx_i}{dt} = f_i(x_1,\ldots,x_n,t),\ i = 1,\ldots,n$$

Solve Differential Equations in Milliseconds!

Berkeley Madonna is arguably the fastest, most convenient, general purpose differential equation solver available today. It is relatively inexpensive and runs on both Windows and Mac OS. Developed on the Berkeley campus under the sponsorship of NSF and NIH, it is currently used by academic and commercial institutions for constructing mathematical models for research and teaching.

Hamner--Now ScitoVation Team Short courses & ….



This second book was more of a how-to manual with specific examples, including computer code

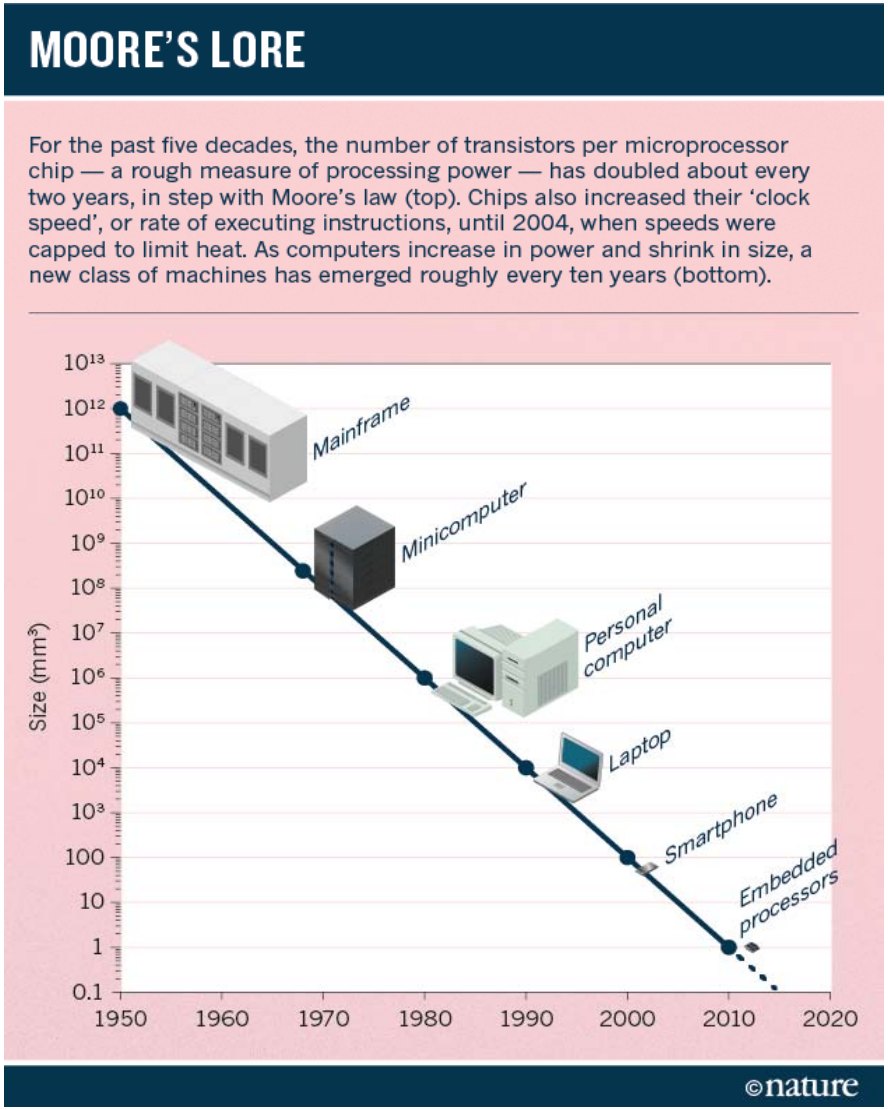
Diverse group of authors world-wide representation.

Kannan Krishnan, a former postdoctoral student, has also led a program at the University of Montreal with a focus on PBPK approaches.

Jeff Fisher at Georgia for 10 years also led a program with a heavy emphasis on PBPK modeling

Resources & Technology

From tedious entry of punch cards across campus on a university mainframe, on to the 8I DEC, then to small personal computers (1986), and now with the enormous computational power on 1 lb portables - astonishing !



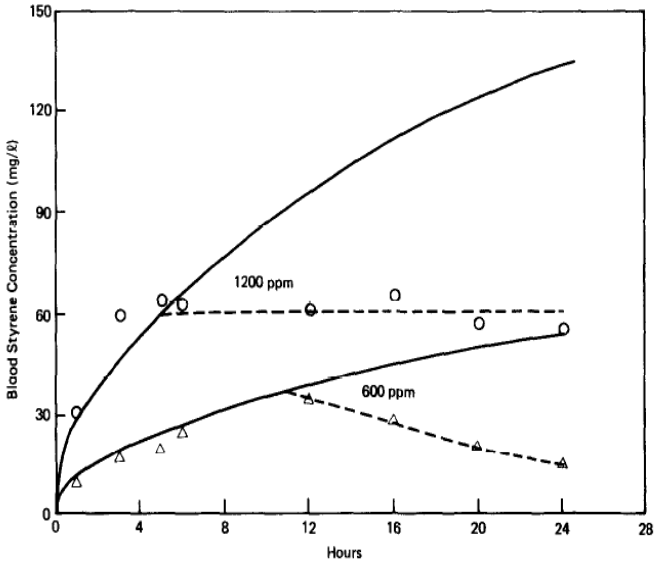
Resources & Technology in The Century of Biology

Part 2: Modeling measures of response

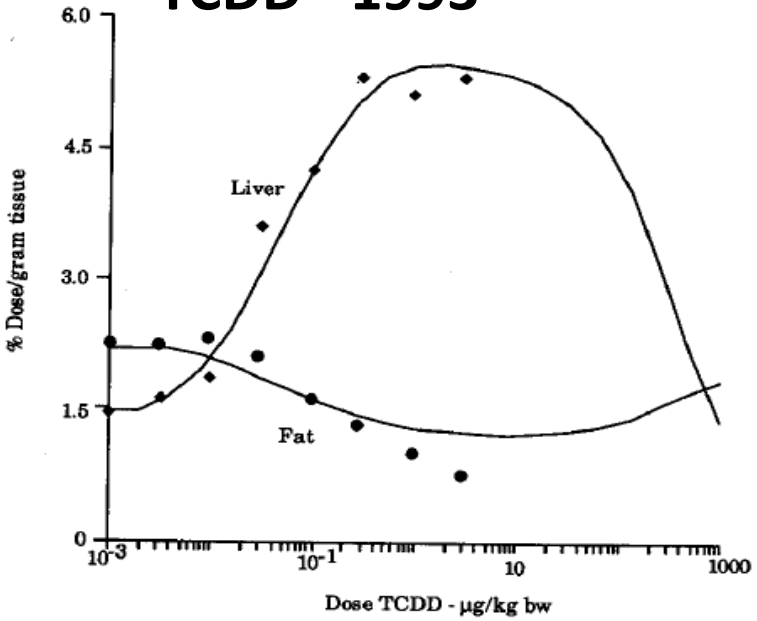
- Time & dose dependent changes with styrene, TCDD
- Appreciating the role of the cell as the responding unit
- NAS report–"Toxicity Testing in the 21st Century: A Vision and A Strategy"
- Moving "the vision" into practice at The Hamner
- Reflections on a long-career–that seems all to short

Styrene - 1984

- Continuous exposures lead to decreases in blood concentrations
- Styrene exposure appears to induce enzymes involved in its metabolism
- Used a time dependent $V_{maxc,t}$



TCDD - 1993

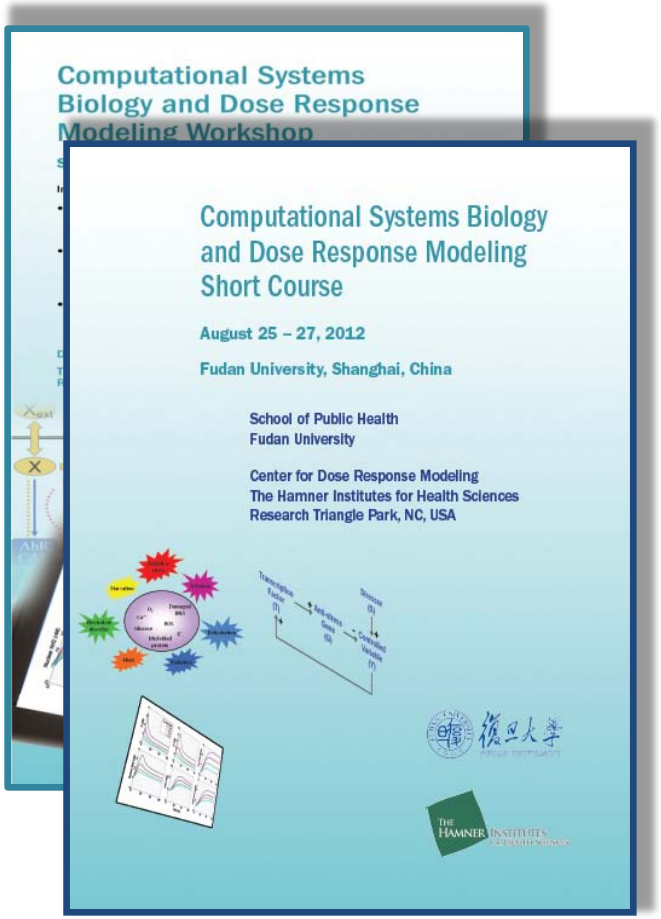


- Presumed there was induction of binding protein in liver
- Al Poland showed it was CYP1A2
- Modeled with Ah binding TCDD and the Ah-TCDD complex binding to dioxin response elements on CYP1A2

Modeling cellular responses:

- Responses occur in a concerted manner–many genes respond together rather than as individual genes competing for limited amounts of activated receptors.
- Responses increasingly seen as all-or-none. A cell either responds completely to the stimulus or remains in its basal state.
- Our Hamner team then worked to understand modern feedback controlled computational modeling of cell signaling pathways using simulation languages focusing on two kinds of processes–going somewhere else and staying in place.

Understanding the “expected” behavior, dynamics, “responses”–a bit like Andersen 1981



Journal of Toxicology and Environmental Health, Part B, 13:253–276, 2010

ISSN: 1093-7404 print / 1521-6950 online
DOI: 10.1080/10937404.2010.483943

Taylor & Francis

COMPUTATIONAL SYSTEMS BIOLOGY AND DOSE-RESPONSE MODELING IN RELATION TO NEW DIRECTIONS IN TOXICITY TESTING

Qiang Zhang[1] Sudin Bhattacharya[1] Melvin E. Andersen[1] Rory B. Conolly[2]

Review

Molecular Signaling Network Motifs Provide a Mechanistic Basis for Cellular Threshold Responses

Qiang Zhang,[1] Sudin Bhattacharya,[1] Rory B. Conolly,[2] Harvey J. Clewell III,[1] Norbert E. Kaminski,[3,4] and Melvin E. Andersen[1]

[1]Institute for Chemical Safety Sciences, The Hamner Institutes for Health Sciences, Research Triangle Park, North Carolina, USA; [2]Integrated Systems Toxicology Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental

open Biology

Ultrasensitive response motifs: basic amplifiers in molecular

OXFORD

SOT | Society of Toxicology
www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 147(2), 2015, 302–316
doi: 10.1093/toxsci/kfv130
Contemporary Review

CONTEMPORARY REVIEW

Adaptive Posttranslational Control in Cellular Stress Response Pathways and Its Relationship to Toxicity Testing and Safety Assessment

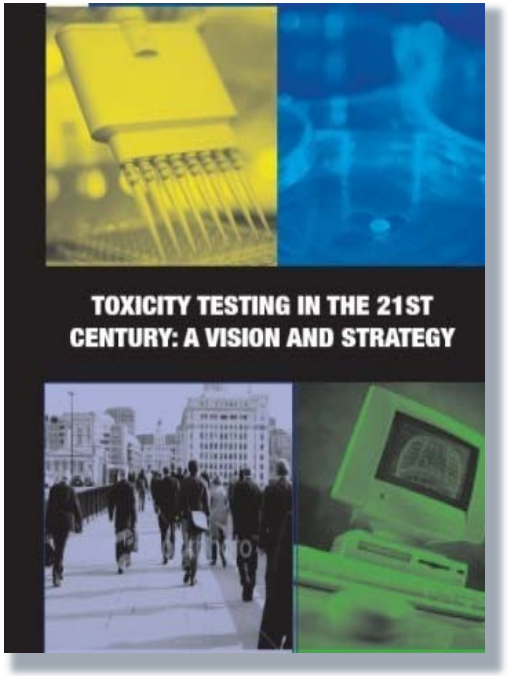
Qiang Zhang,*,[1] Sudin Bhattacharya,* Jingbo Pi,† Rebecca A. Clewell,* Paul L. Carmichael,‡ and Melvin E. Andersen*

*Institute for Chemical Safety Sciences, The Hamner Institutes for Health Sciences, Research Triangle Park, North Carolina 27709; †School of Public Health, China Medical University, Shenyang, China; and ‡Unilever, Safety and Environmental Assurance Centre, Colworth Science Park, Sharnbrook, Bedfordshire, UK

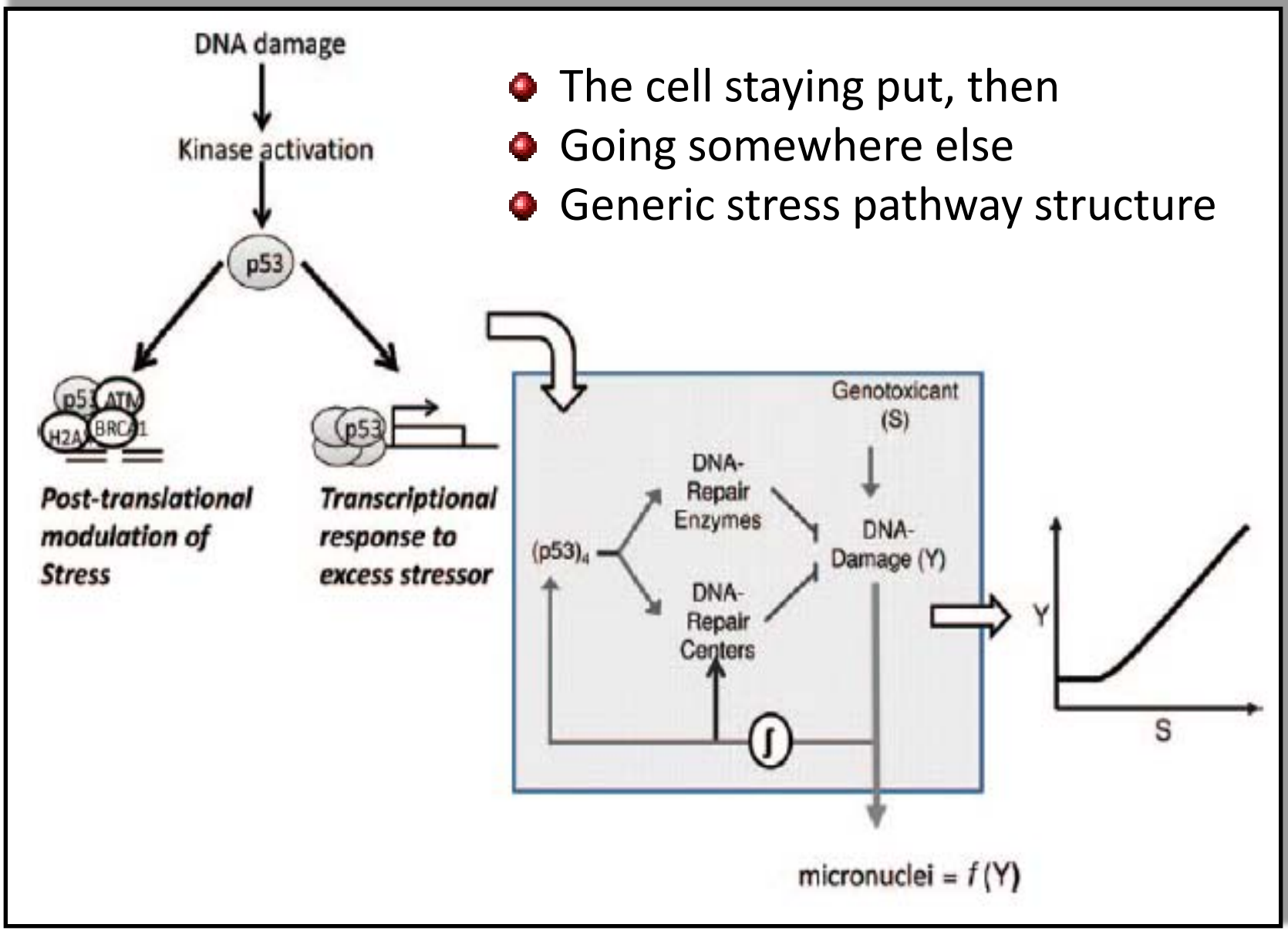
[1]To whom correspondence should be addressed at Institute for Chemical Safety Sciences, The Hamner Institutes for Health Sciences, Research Triangle Park, North Carolina 27709. Fax: 1-919-558-1300. E-mail: qzhang@thehamner.org

Moving to Application of these cell-based response tools

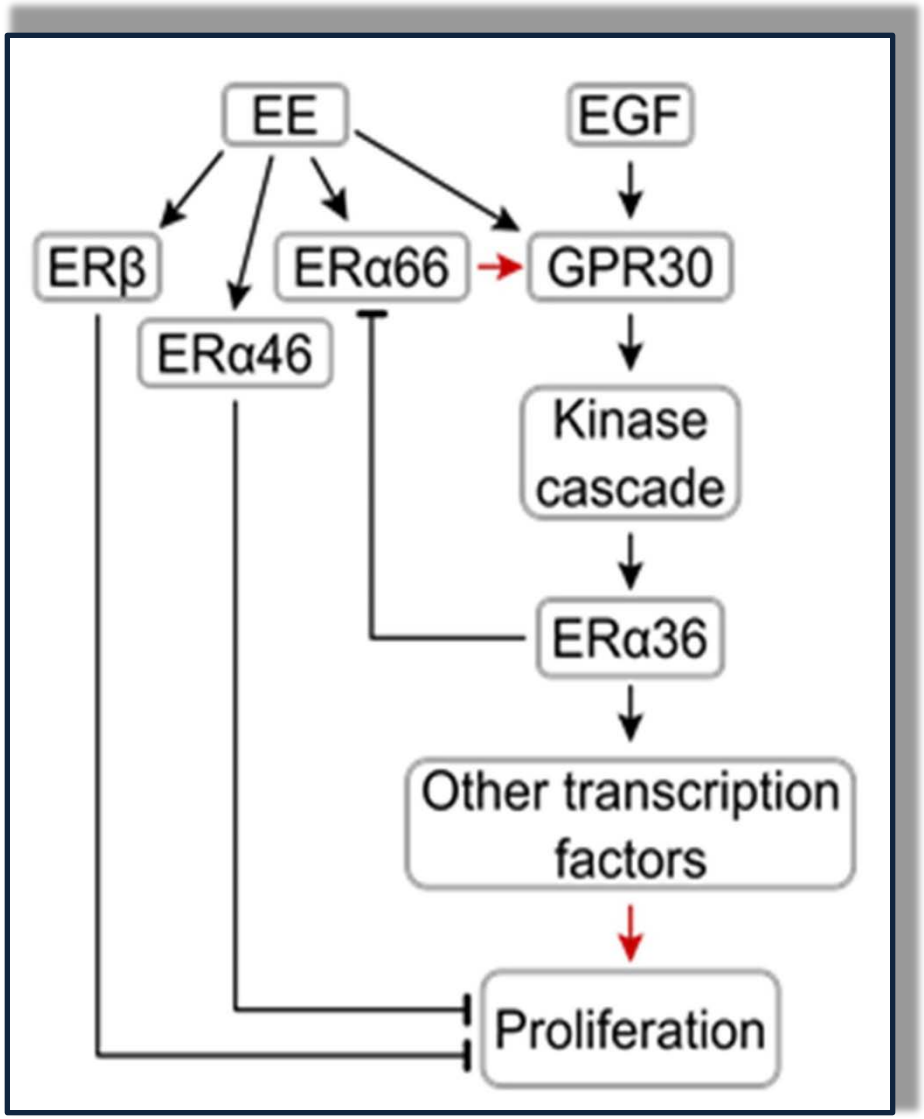
- A 2007 NAS committee report–**another career altering event**
- Testing in human cells, mode of action based in vitro assays, computational systems biology and *in vitro* and *in vivo* modeling for extrapolation
- **Hamner team moved to develop case studies with known pathways to show these TT21C approaches in practice**



DNA-Damage Pathways



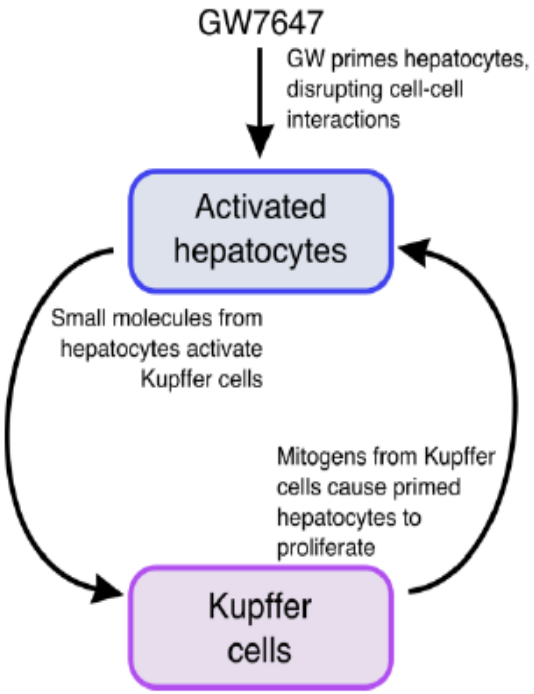
- The cell staying put, then
- Going somewhere else
- Generic stress pathway structure

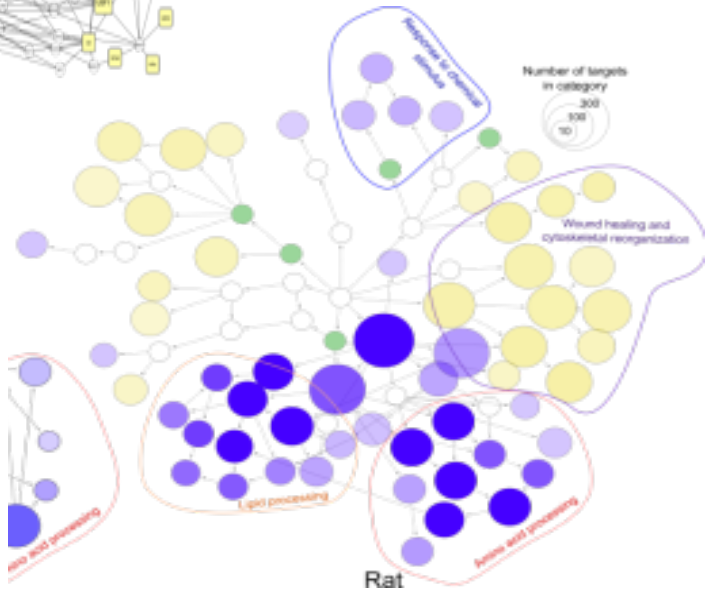


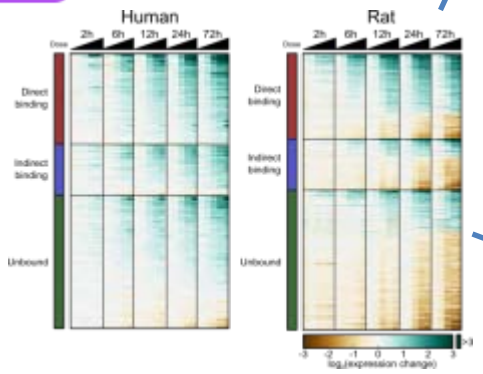
Estrogen Signaling:

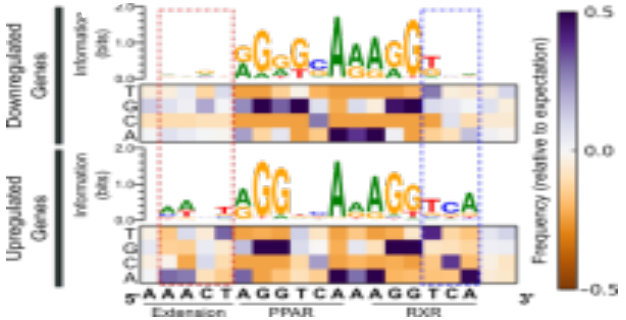
- Going somewhere else– proliferation
- Coordinate control of a suite of receptor processes that appear to be related to both activating and limiting the response
- External input–EGF–serves as a paracrine mediator
- Possibly, a more generic nuclear receptor mediated pathway

PPARα Signaling









Treat for me at least–finding out the basis for "incidence–dose relationships at the cellular level"–woof, woof!

What are the advantages of modeling in toxicology?

CONTEMPORARY ISSUES IN TOXICOLOGY

Applying Simulation Modeling to Problems in Toxicology and Risk Assessment—A Short Perspective

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1. Codification of facts and beliefs (organize information)
2. Expose contradictions in existing data/beliefs
3. Explore implications of beliefs about the system
4. Expose serious data gaps
5. Predict response under new conditions
6. Predict parameter values for "inaccessible" parameters
7. Identify essentials of system structure
8. Provide representation of current state of knowledge
9. Suggest and prioritize new research

The joy of finding out that you are wrong!

What's next? Using these new approaches for safety assessments

There will be a need to get the word out–**to educate**–and develop next-generation practitioners

We need to complete another short-course!

Hamner/ScitoVation Team (2002-2016)

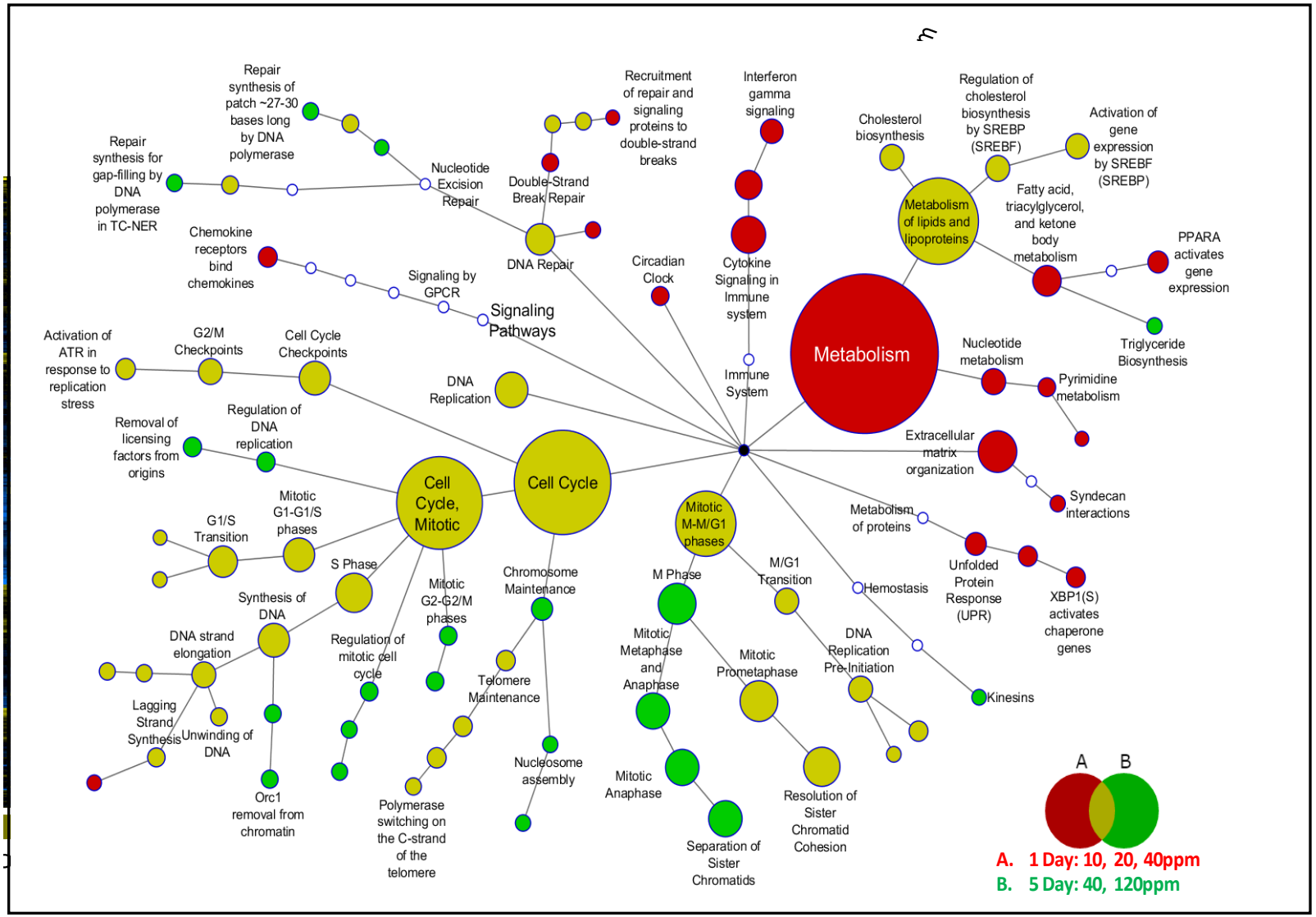
- Rory Conolly
- Rusty Thomas

- Harvey Clewell
- Barbara Wetmore
- Miyoung Yoon

- Qiang Zhang
- Sudin Bhattacharya
- Courtney Woods

- Rebecca Clewell
- Patrick McMullen
- Bin Sun
- Michelle Miller
- Chad Deisenroth

Some Reflections on my “unanticipated” career as a toxicologist–be careful of the baggage we carry



Did I have the points correct earlier?

Opportunities

Circumstances

Persistence

Resources & Technology

Staff, colleagues, supervisors, family, friends

Friends are really hard to do without– they enrich our lives

A great deal of good fortune and some hard work

Some, more than a small degree of good fortune