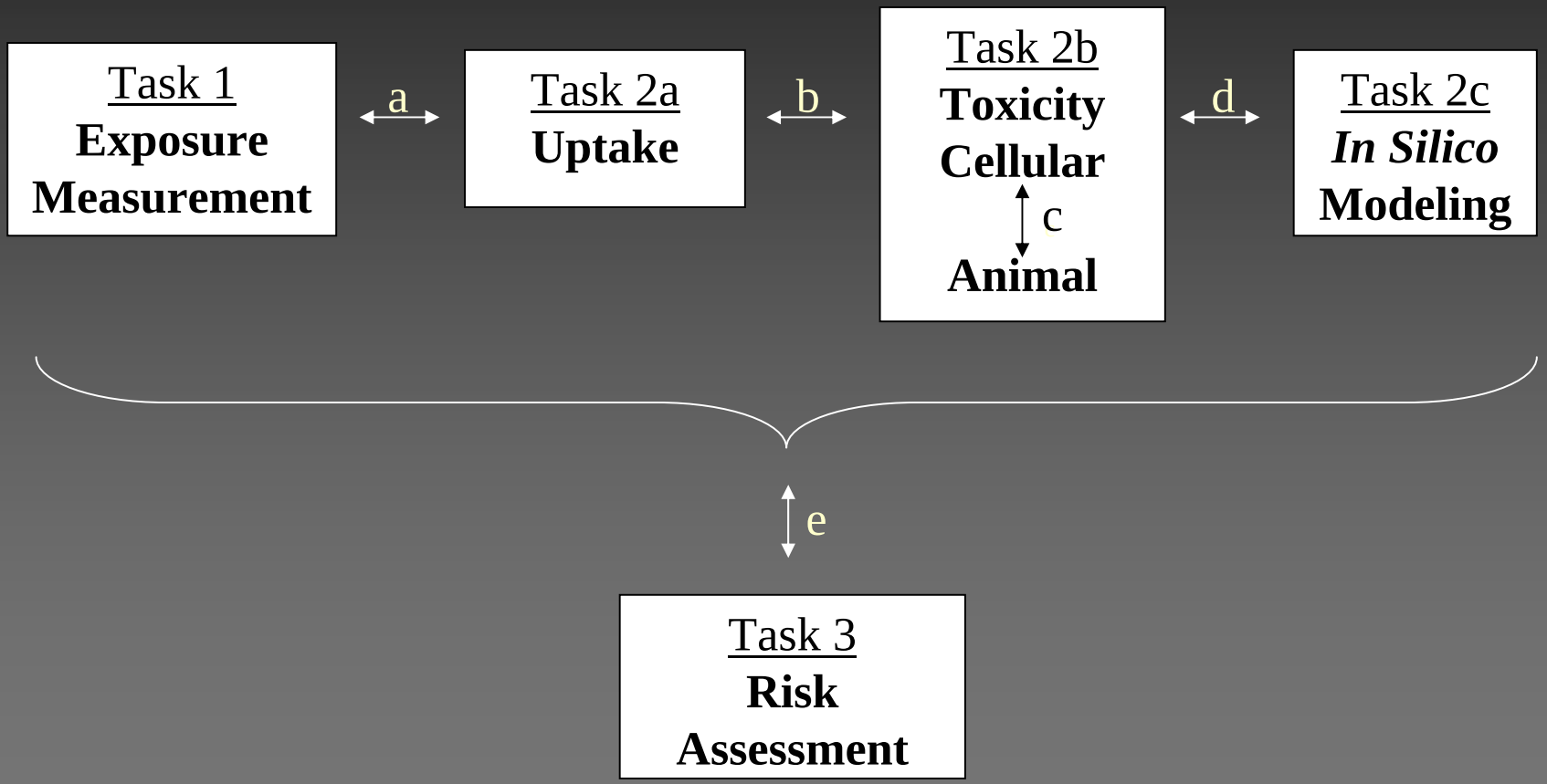
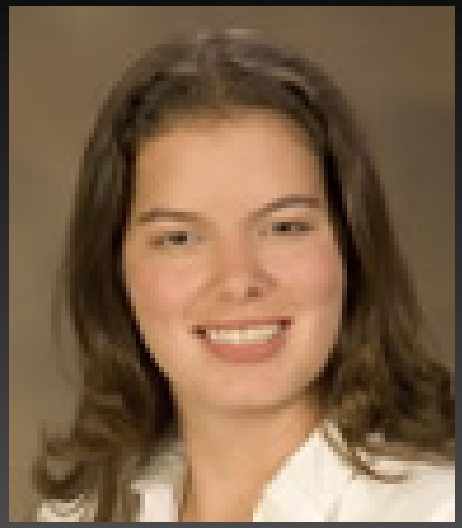
Environmental Health and Nanomaterials: Development of exposure analysis, toxicity tests, and predictive risk assessment methods

Clark Lantz
Department of Cell Biology and Anatomy
Southwest Environmental Health Science Center
University of Arizona
Tucson, AZ

Strategy for determining risk

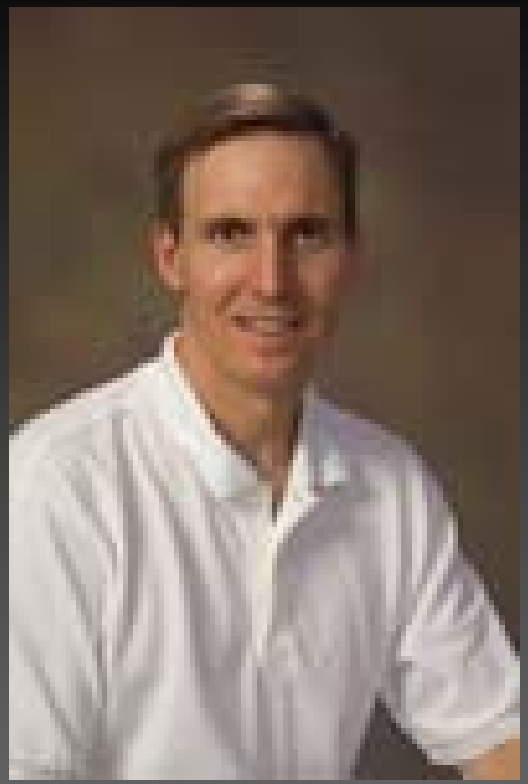




Paloma Beamer, PhD
Community, Environment and Policy
COPH, University of Arizona

Pierre Herckes, PhD
Chemistry and
Biochemistry, ASU

Paul Blowers, PhD
Chemical and Environmental Engineering
University of Arizona



Jeff Burgess, MD, MS, MPH
Environmental & Occupational Health
COPH, University of Arizona

Scott Boitano, PhD
Physiology
COM
University of Arizona

Occurrence of nanoparticles in the environment (Herckes)

Task 1
Exposure Measurement

Two main challenges to the determination of engineered NPs in the environment

very low mass concentrations of NPs are expected
detection at trace and ultra-trace levels (ppb and below)
→ need for highly sensitive techniques

complexity of environmental samples (air/aerosol or water)
presence of natural NPs (colloids) and incidental NPs (combustion)
presence of NP components in different (non-NP) forms

→ need for highly specific methods to
differentiate between NP component and natural component
differentiate by NP size (single NP or aggregate)
differentiate between engineered NP and natural/incidental NPs

Occurrence of nanoparticles in the environment (Herckes)

Most promising analytical solutions involve coupling of separation techniques with highly sensitive mass spectrometry

- C60 and C70 fullerenes using liquid chromatography coupled to mass spectrometry (HPLC-MS/MS)
- metal and metal oxide NPs using field flow fractionation or size exclusion chromatography coupled to inductively coupled plasma mass spectrometry (FFF-ICP/MS or SEC-ICP/MS)

For fate studies, the use of stable isotopes is explored for environmental and biological tracking of engineered NP material

HOWEVER: Currently most analytical techniques are derived from quality control in synthesis or production processes featuring

- detection limits orders of magnitude higher than in the environment
- overly simple sample matrices

Strong need to further refine analytical tools

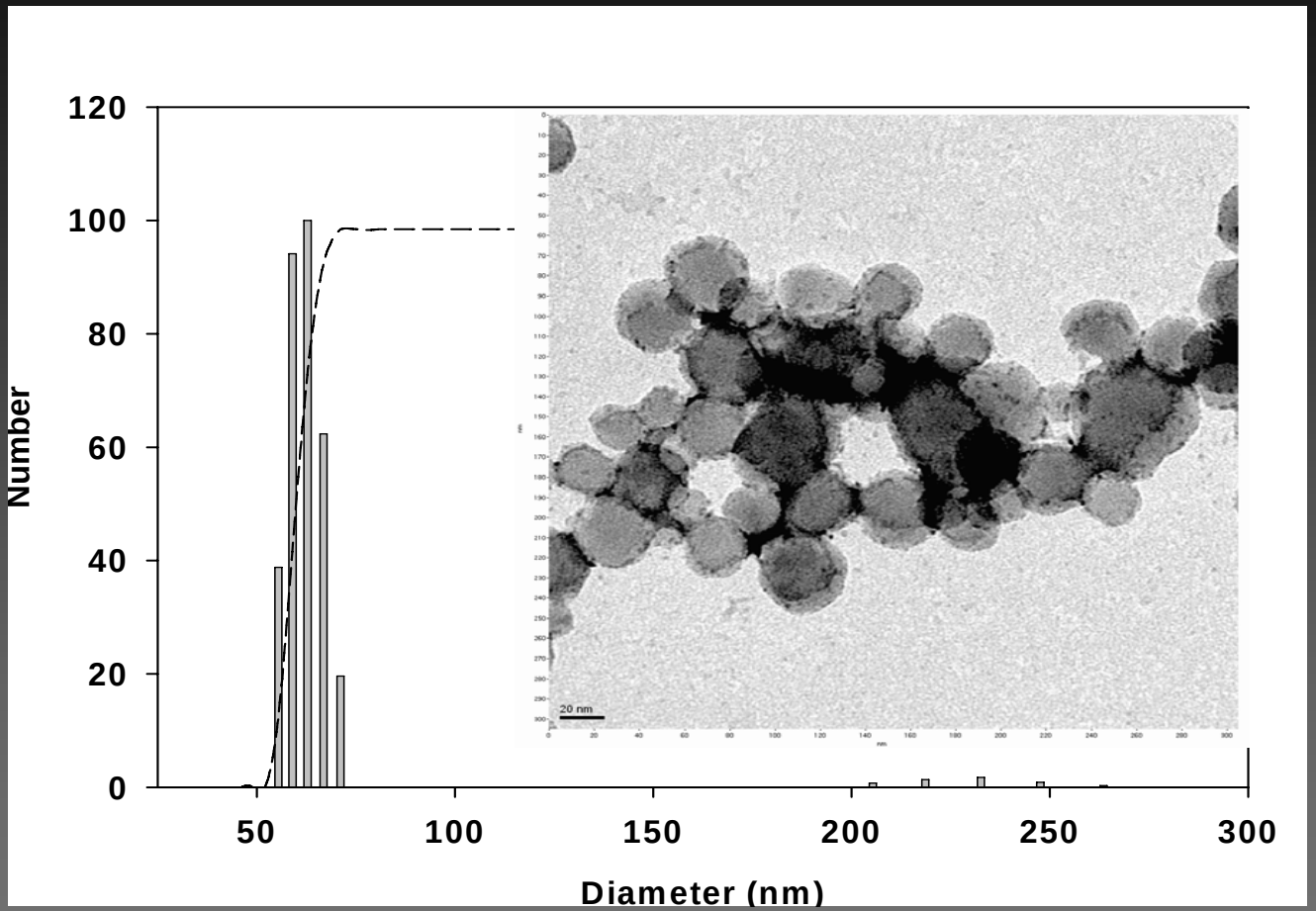


Figure 1 Dynamic light scattering size distribution and inset of Transmission Electron Microscope (TEM) image of soluble n-C_{60} fullerene aggregates

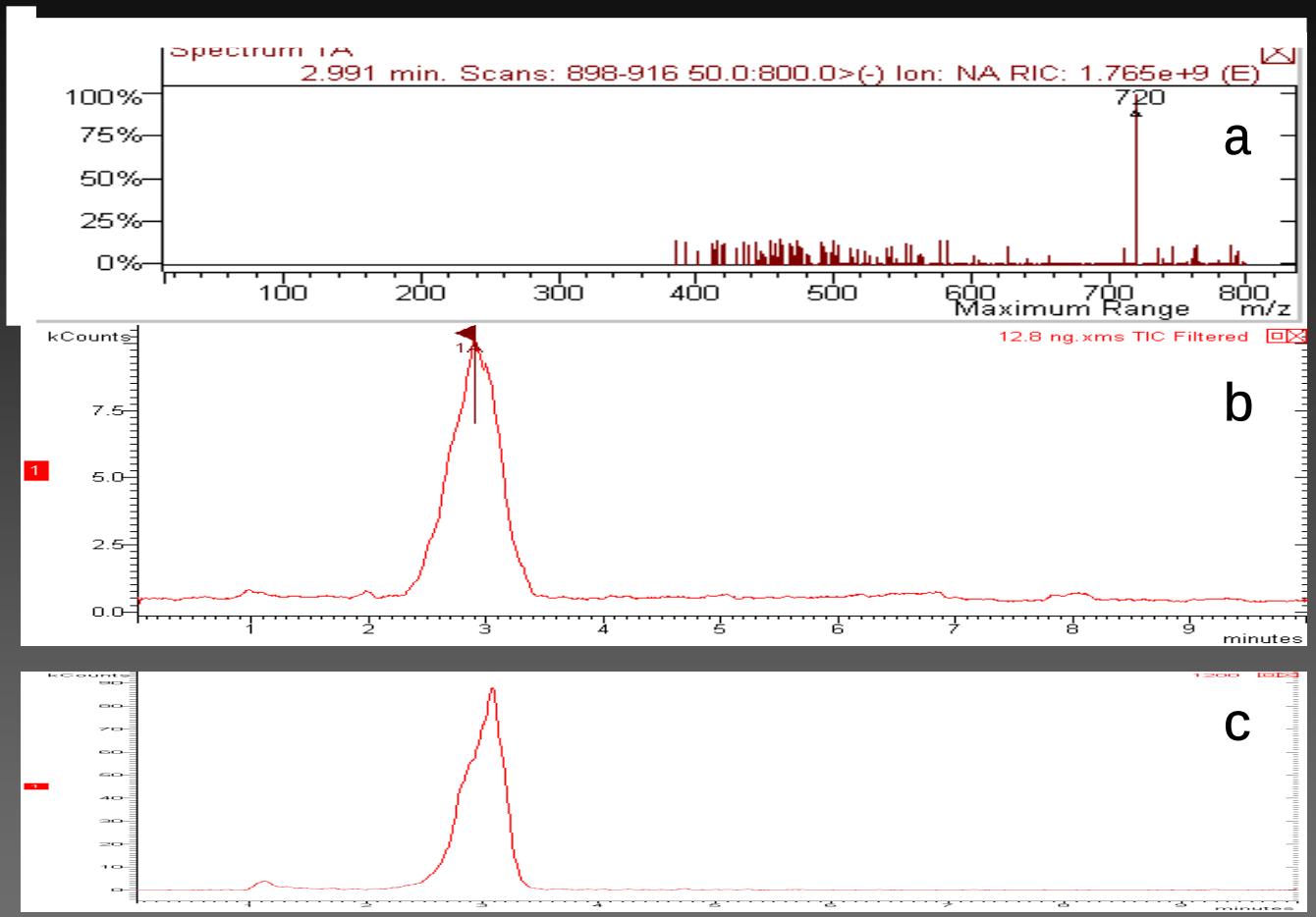
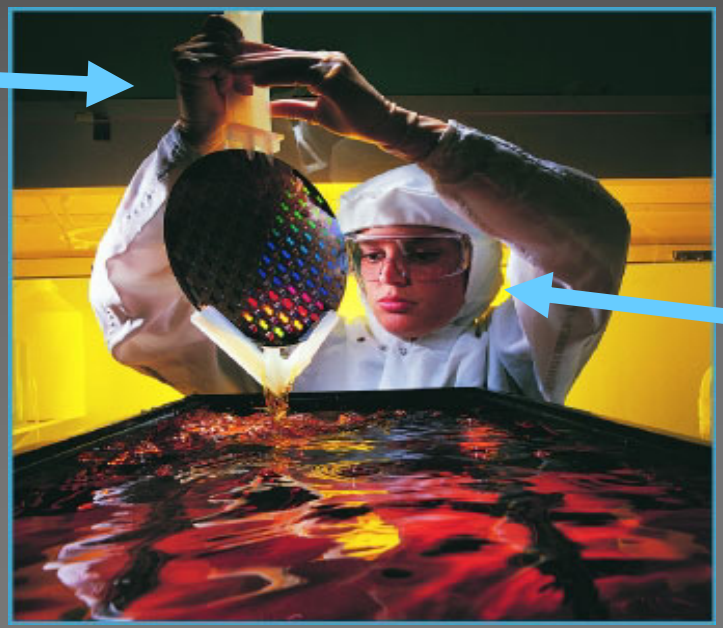


Figure 2 Total ion chromatography of fullerenes by LC/MS. a) TIC of C_{60} in water, b) LC/MS chromatogram of 12.8 μg/L of n-C_{60} in toluene stock solution, c) LC/MS chromatogram of 10 μg/L of C_{60} after salt-toluene extraction from water.

Strategy for determining risk

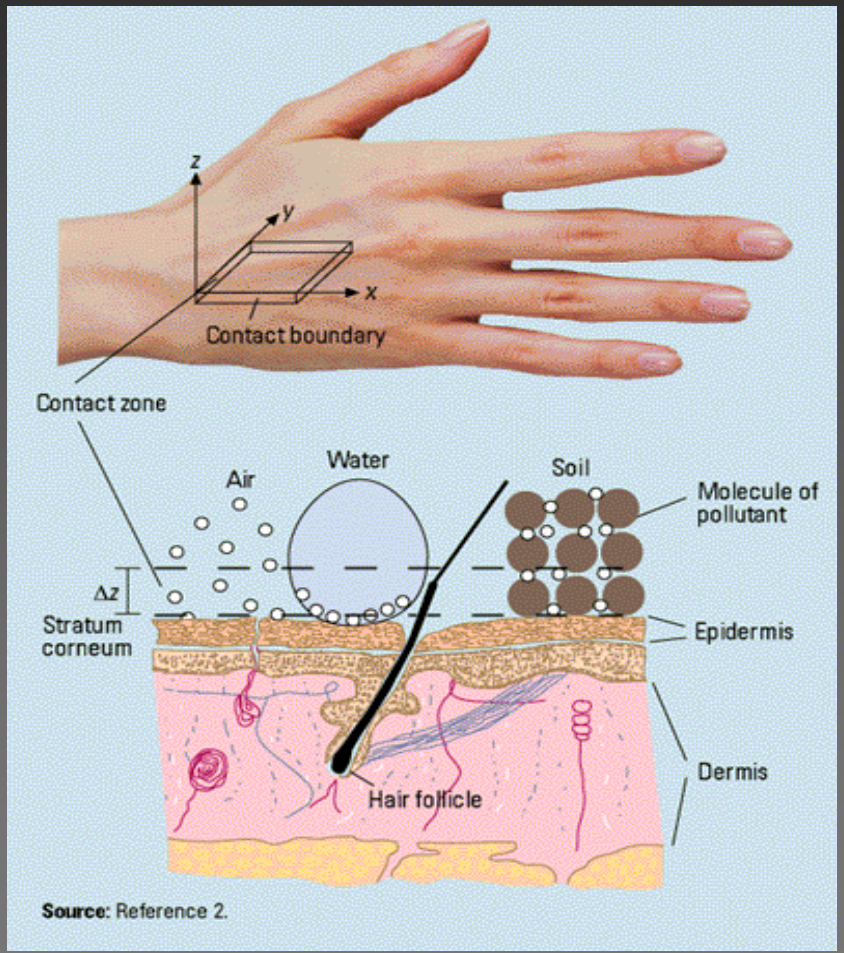
Task 1 **Exposure Measurement**	a	Task 2a **Uptake**

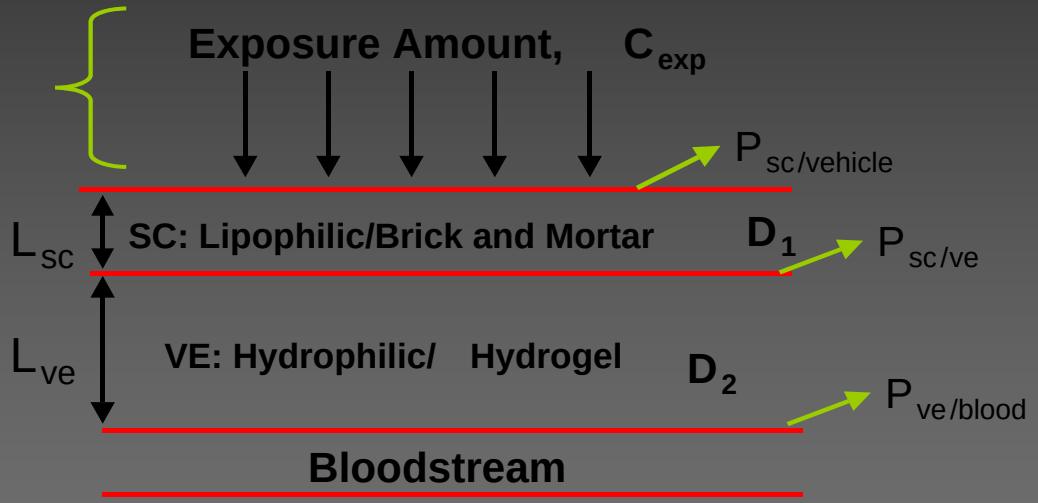
Dermal
- air immersion
- liquid contact
- aerosol deposition



Inhalation

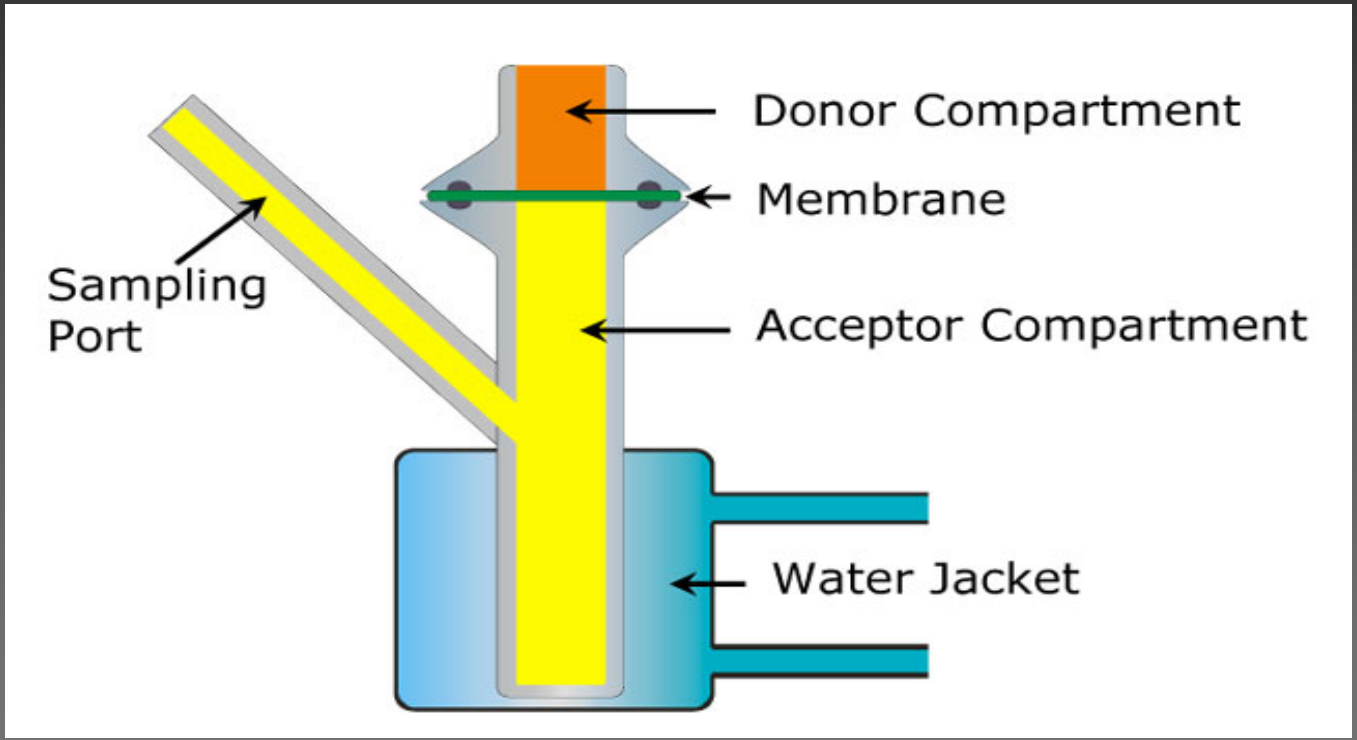
Dermal exposure and absorption (Beamer)





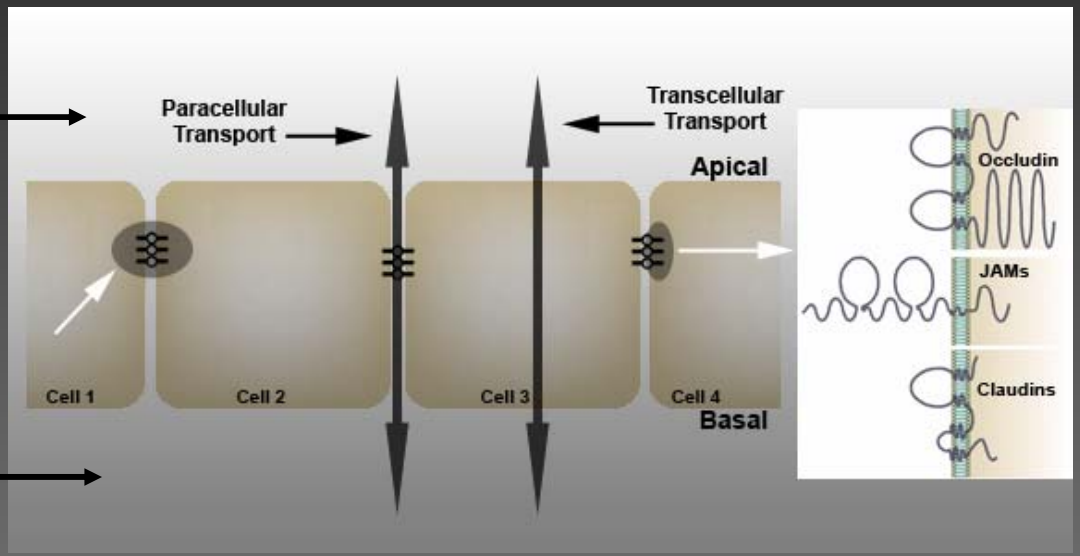
Adapted from Zartarian and Leckie (1998).

Dermal uptake measurement



Transport across airway epithelial cells

Airway



Sub-epithelium

Determine uptake across the barrier

This will also be a toxicological measurement

Measurement system is similar to dermal uptake

Strategy for determining risk

Use both *in vitro* and whole animal models to assess toxicity (Beamer, Boitano, Burgess, Lantz)

Examples of types of measurements that can be made to determine sites and extent of toxicity

- Arsenic is widespread in the environment
  - Inhalation
  - Water
- Epidemiological studies implicate arsenic as a carcinogen
- Important occupational exposures

Role of models for toxicity studies

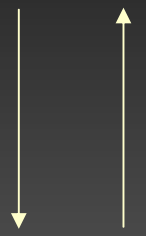
Humans

1. Epidemiological Studies

Arsenic exposure and increased lung cancer and chronic lung disease

5. Population/Epidemiology Studies

Biomarkers – exposure/effect
Prevention
Intervention



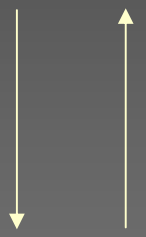
Animals

2. Animals Models

Arsenic altered gene expression and phenotype

4. Mechanisms tested

In vivo, develop biomarkers and potential interventions



Cell lines

3. Mechanistic Studies

Cell lines, cellular-molecular studies

Mechanisms of action - hypotheses formulated.

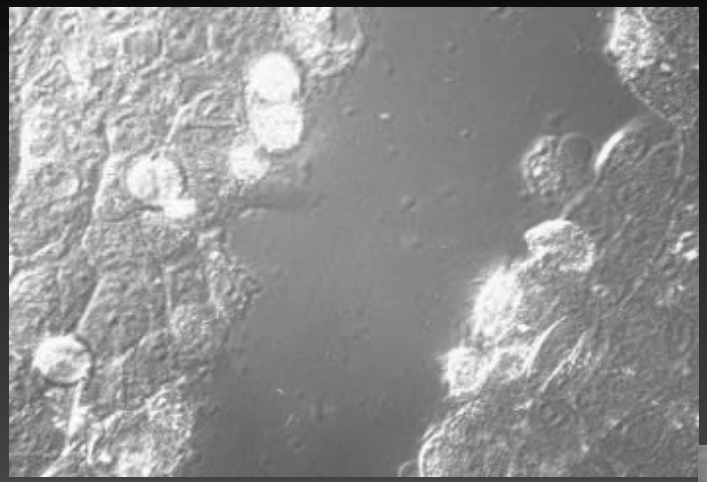
In vitro measurements related to inhalation of NPs

Used an *in vitro* system to begin to address this question.

A transformed human bronchial epithelial cell line, 16HBE140-, were used to analyze the effect of arsenic on cellular and tissue function

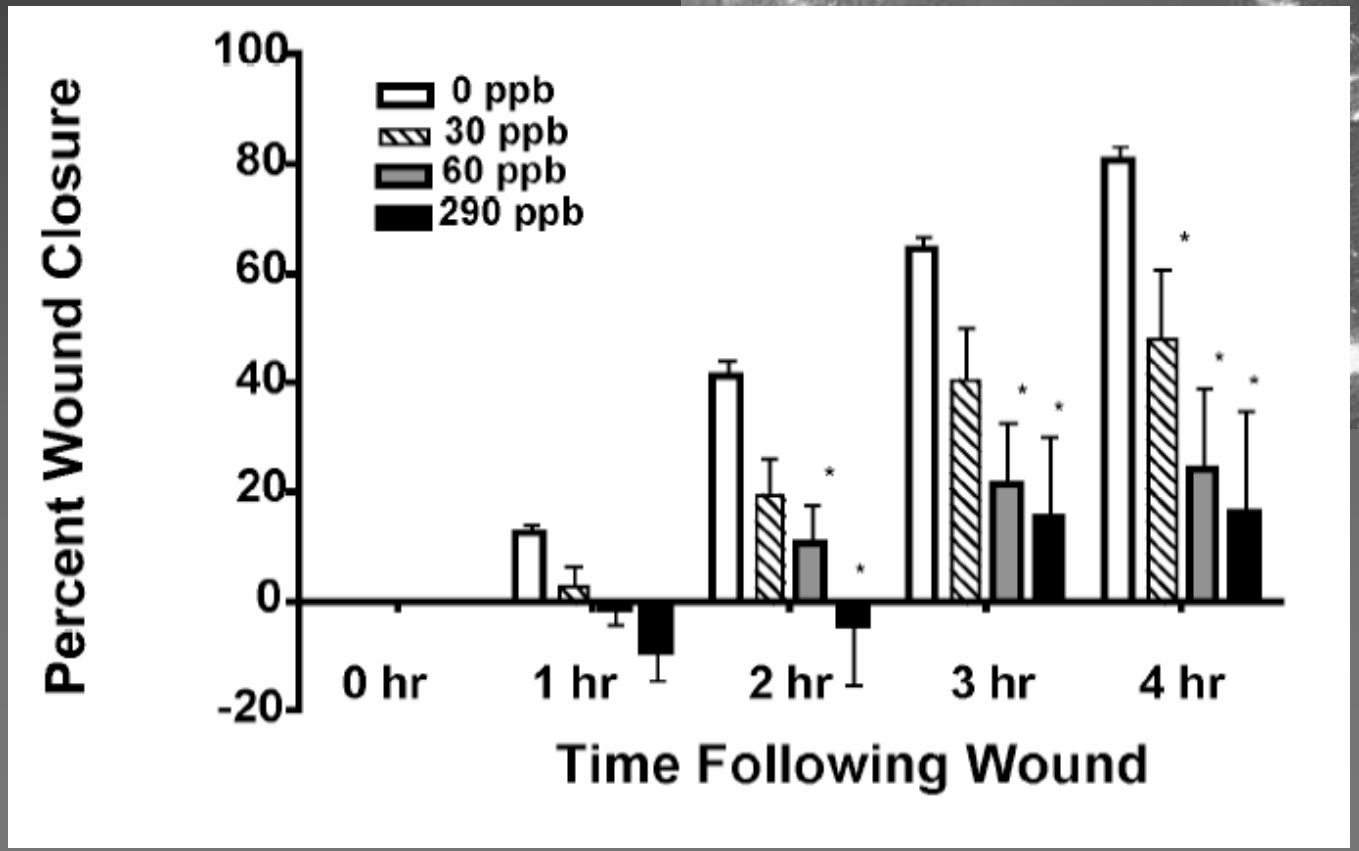
Measure directly the ability of the compounds to cause cell death
Measure function

- Repair of “wounds”
- Alteration in epithelial barrier function



“Scratch wound healing” model

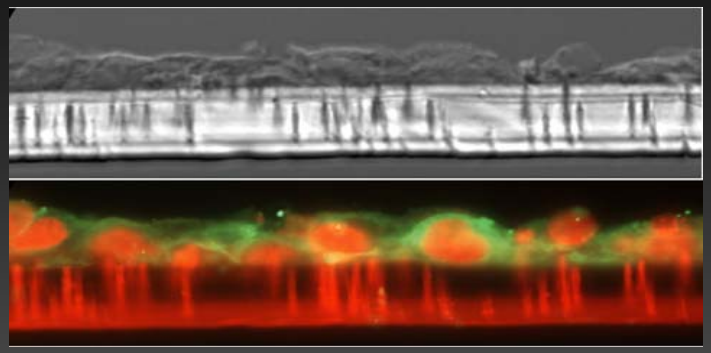
Cells were grown to confluence, exposed to arsenic for 24 hrs and then plate was scraped to produce “wound”

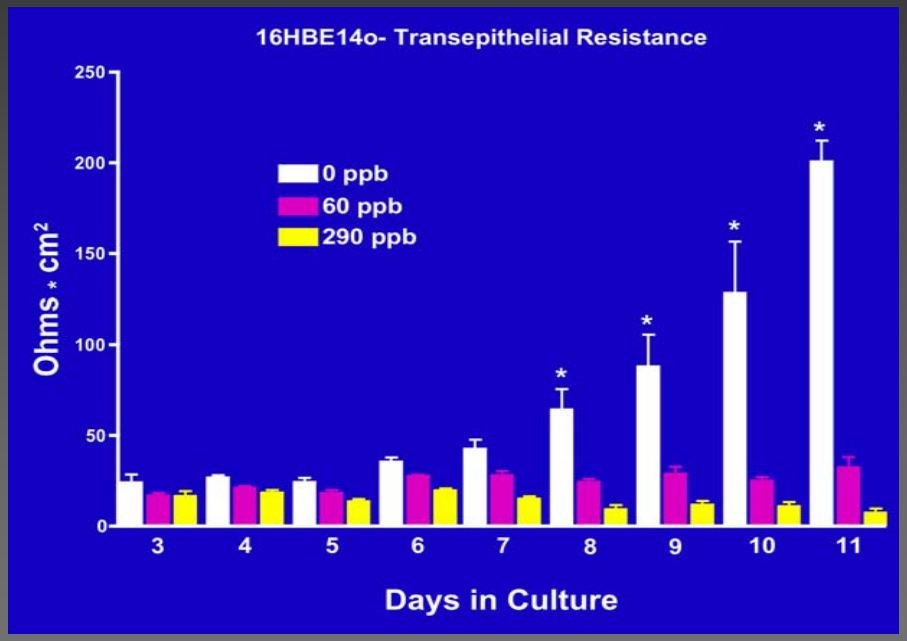


290 ppb

Arsenic effects on barrier function

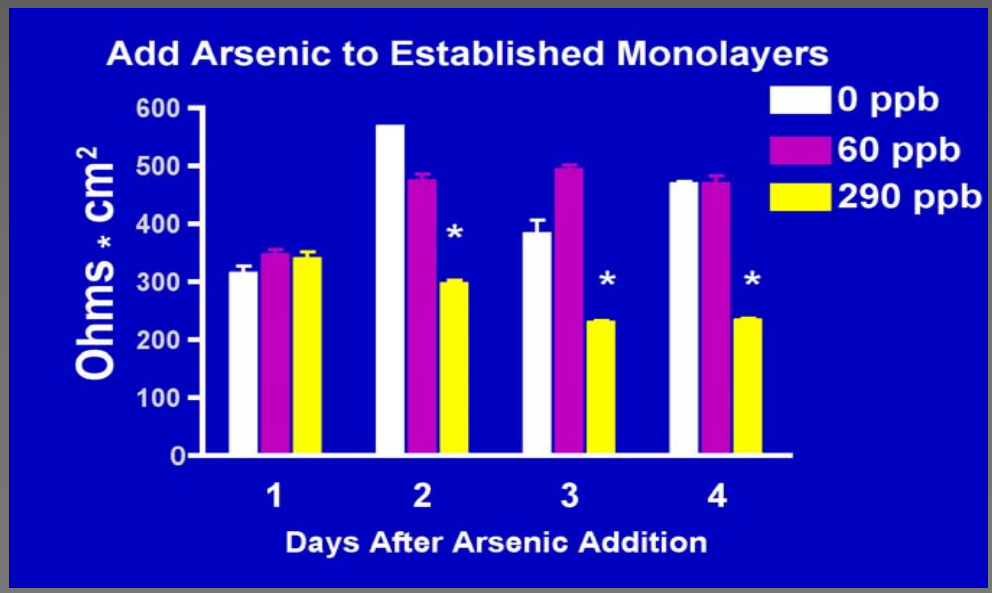
- Grew Cells to Confluence on Nuclepore Filters +/- arsenic in the growth medium
- Monitored transepithelial resistance (TER) over time





- Arsenic prevents establishment of a functional barrier
- **Arsenic can contribute to breakdown of a functional barrier**

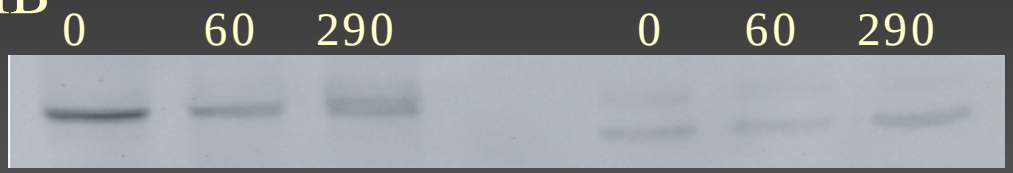
- Grew Cells to Confluence on Nuclepore Filters without arsenic to establish an TER
- Added arsenic to growth medium
- Monitored transepithelial resistance (TER)



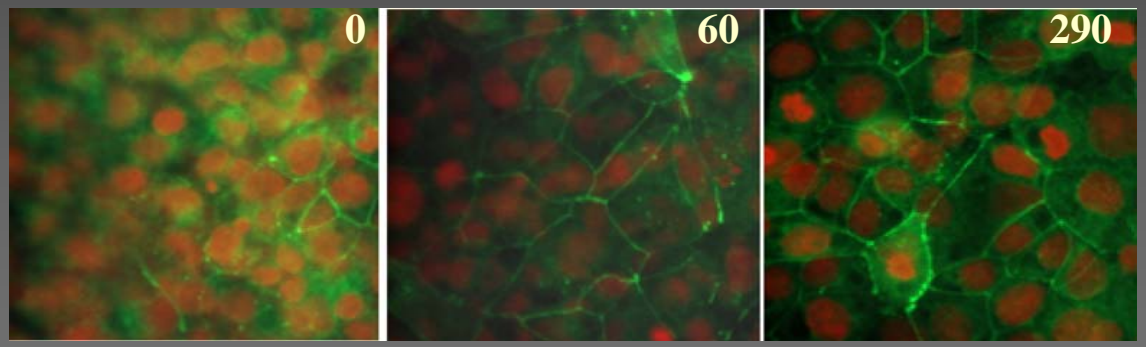
Arsenic effects on tight junction proteins

- Grew Cells to Confluence on coverslips and/or flasks
- Added arsenic to medium for 3-5 days
- Assayed tight junction proteins/mRNA expression using
  - Immunoblot
  - Immunocytochemistry
  - qRT-PCR

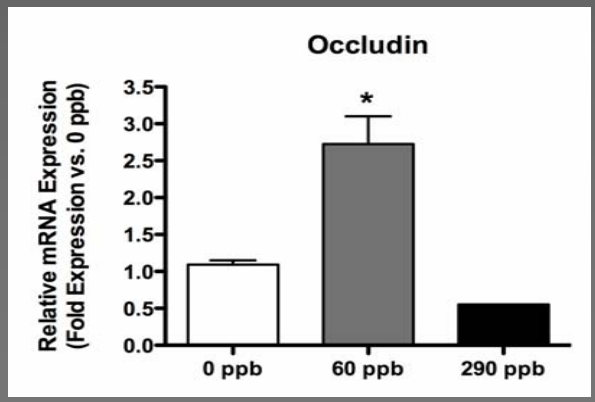
Occludin IB



Occludin IC



Occludin qRTPCR



Occludin is altered by arsenic

Arsenic-induced alterations *in vivo*

Arsenic ingestion

- Adult male mice were given arsenic in the drinking water (0, 10 or 50 ppb)
- Animals were exposed for up to 8 weeks
- BD Powerblot
- 2-D gel and MS protein identification
  - Whole lung
  - Lung lavage fluid
  - Airway epithelial specific

- Wound repair model

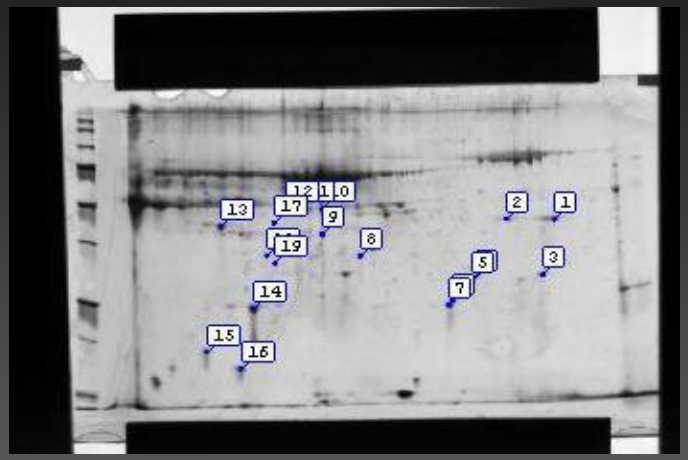
Protein isolated from whole lungs of mice that were exposed to 50 ppb for 8 weeks.

Protein was analyzed by BD Powerblot (~1000 validated antibodies)

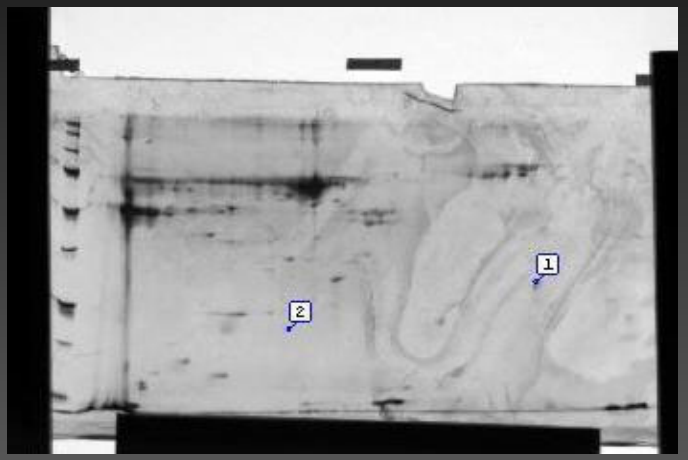
Curated data mining program
- Disease
  - Cancer
  - Wound healing
- Process
  - Cell motility

Protein	Change in expression
Acetylcholinesterase	0.30
Hic-5 (TGF-β1 induced transport)	0.28
Rac1	5.82
Syntaxin 8	present in cont - absent in As
CapZ α	0.36
Stat3	0.52
Calretinin	0.20
Caspase-3	0.23
Melusin	0.10
β-Catenin	0.66
4.1N	2.65
DMPK	0.39
Gelsolin	0.52
IKKγ/NEMO	0.58
Nucleoporin p62	0.32
OPA1	0.32
Rab4	2.23
RCC1	2.65

Proteomics analysis of soluble BALF proteins



Control



50 ppb arsenic

<u>DOWN REGULATED</u>

RAGE (receptor for advanced glycation end products)

GST omega 1-1 (arsenic metabolizing enzyme)

Alpha-1 antitrypsin (important in development of emphysema)

Apolipoprotein A-I and IV

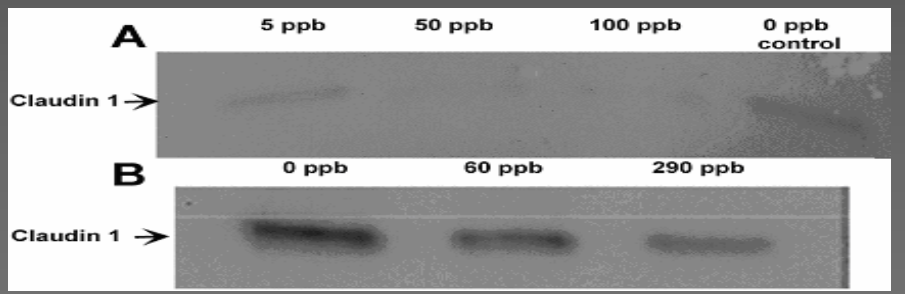
<u>UP REGULATED</u>

Peroxiredoxin-6

Enolase-1

MUDPIT analysis

- BALF
  - 44 proteins identified
    - Included all that were studied on 2-D gels
    - 80% upregulated
- Airway epithelial specific proteins
  - 221 proteins identified
    - 126 downregulated
    - 30 upregulated
    - 55 unchanged

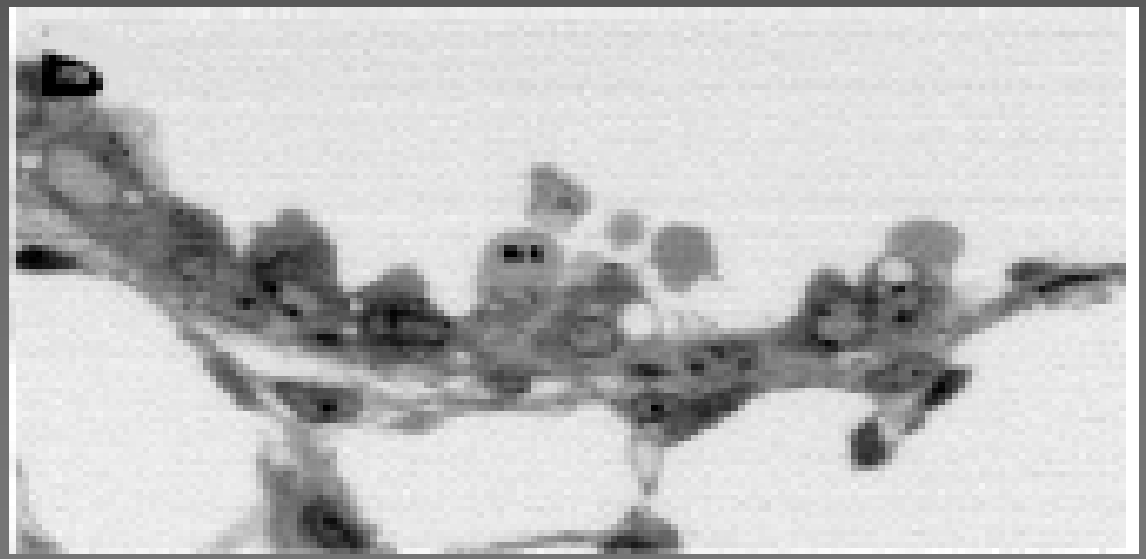


- Analysis with a curated data based found that 20% were associated with wounding and wound healing

In vivo model of wound healing

- Use a naphthalene injury model
  - Selectively kills Clara cells in distal airway
  - Has been used to study wound repair
- Adult mice given 50 ppb arsenic for 4 weeks
- IP injection of NA
- Wait to weeks to look at repair

Control

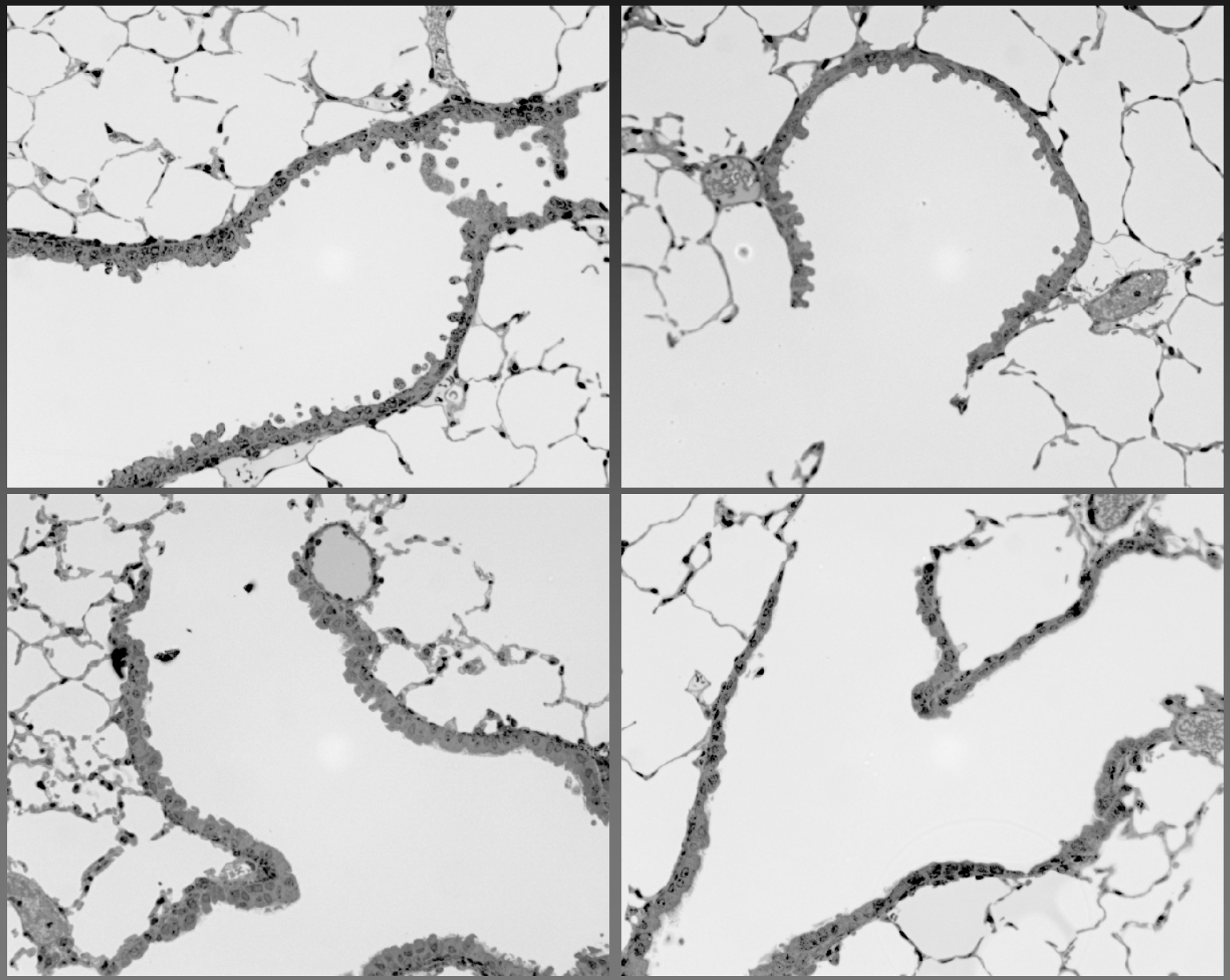


Naphthalene exposed

In vivo wound repair model

Arsenic

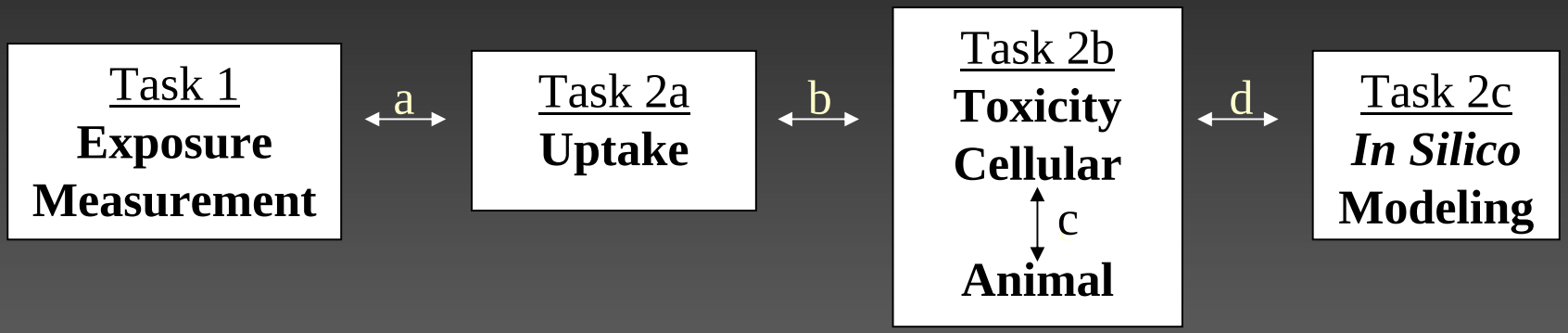
Naphthalene



Key endpoints of toxicity

- Epithelial barrier function
- Cell death
- Inhibition of wound repair
- Alterations in pulmonary function
- Alterations in protein expression
  - Lung lining fluid
  - Airway epithelial cells
- In addition – using stable isotope labeled NP, can study uptake and fate in whole animals
- Data will be applicable for studies in humans

Strategy for determining risk



Correlating Toxicity with Physical and Electronic Parameters – Quantitative Structure Activity Relationships (Blowers)

Use of highest occupied molecular orbital and lowest unoccupied molecular orbital (HOMO-LUMO) energy gap to predict toxicity

Examples include :

correlations of growth inhibition in rat embryos at different dosages with octanol/water partition coeffients and the HOMO-LUMO energy gap for phenolic compounds (Zhang, et al., (1998)).

skin sensitization correlations with electrostatic potentials and the HOMO-LUMO energy gap for a wide range of organic compounds (Miller, et al., (2005)).

light induced toxicity correlated with excited state singlet and triplet energies, and the HOMO-LUMO energy gap for polyaromatic hydrocarbons (Laszlo, et al, (2006))

predicting biodegradability correlated with the HOMO energy and molecular weight for acid dyes (Yin and Li (2007))

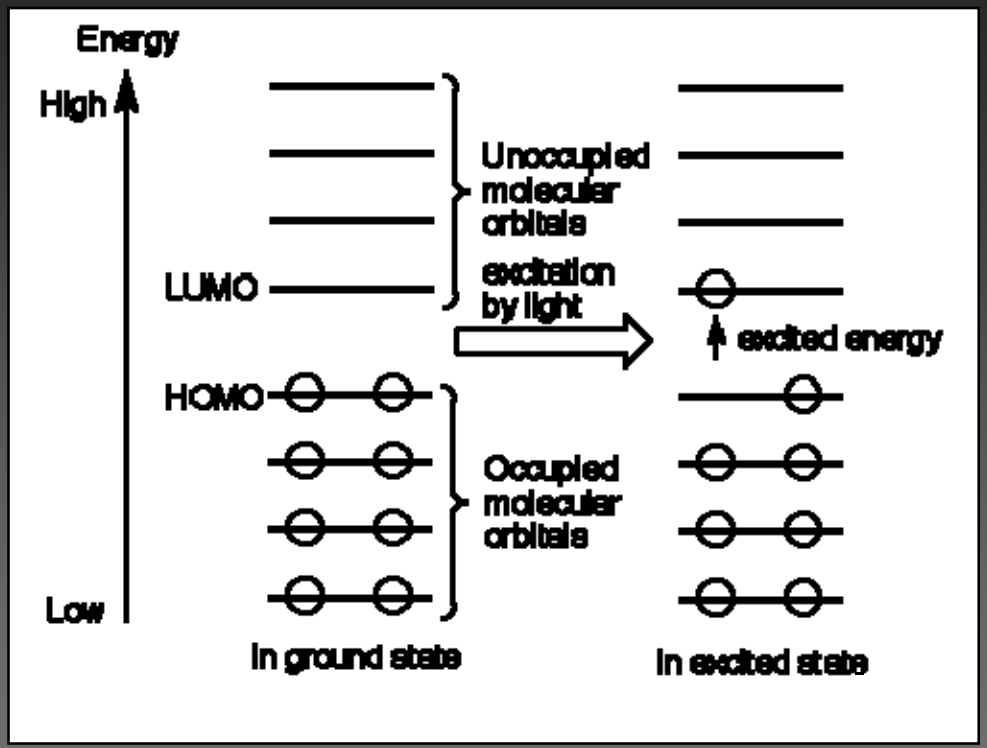
aquatic toxicity of pharmaceuticals correlated with the HOMO-LUMO energy gap (Kim, et al., (2007))

HOMO-LUMO Gap and possibility of performing computations on “large” nano-structures

The HOMO-LUMO gap is easy to calculate for molecular structures.

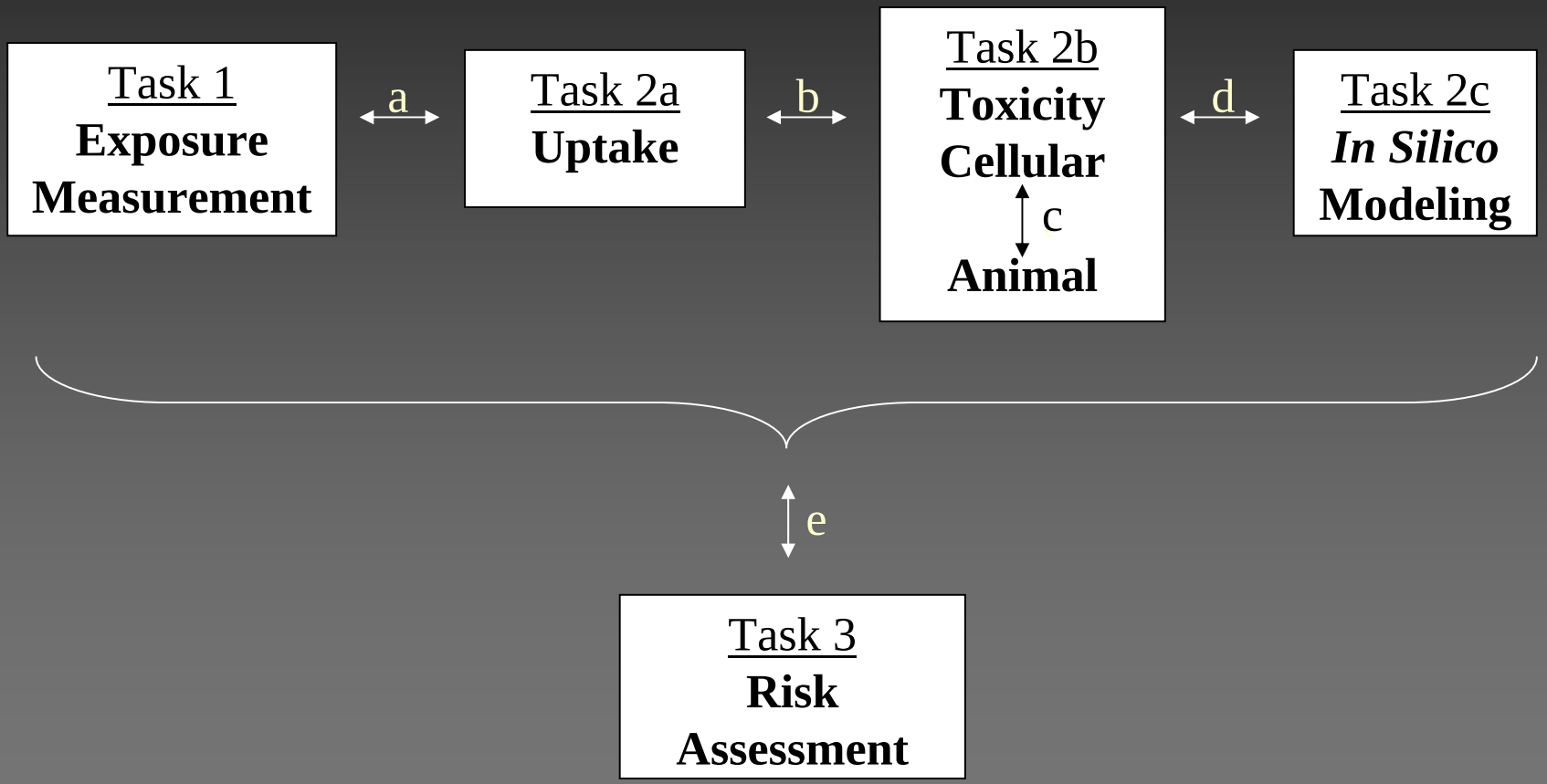
Semi-empirical methods are robust and very fast so even very large species can be investigated.

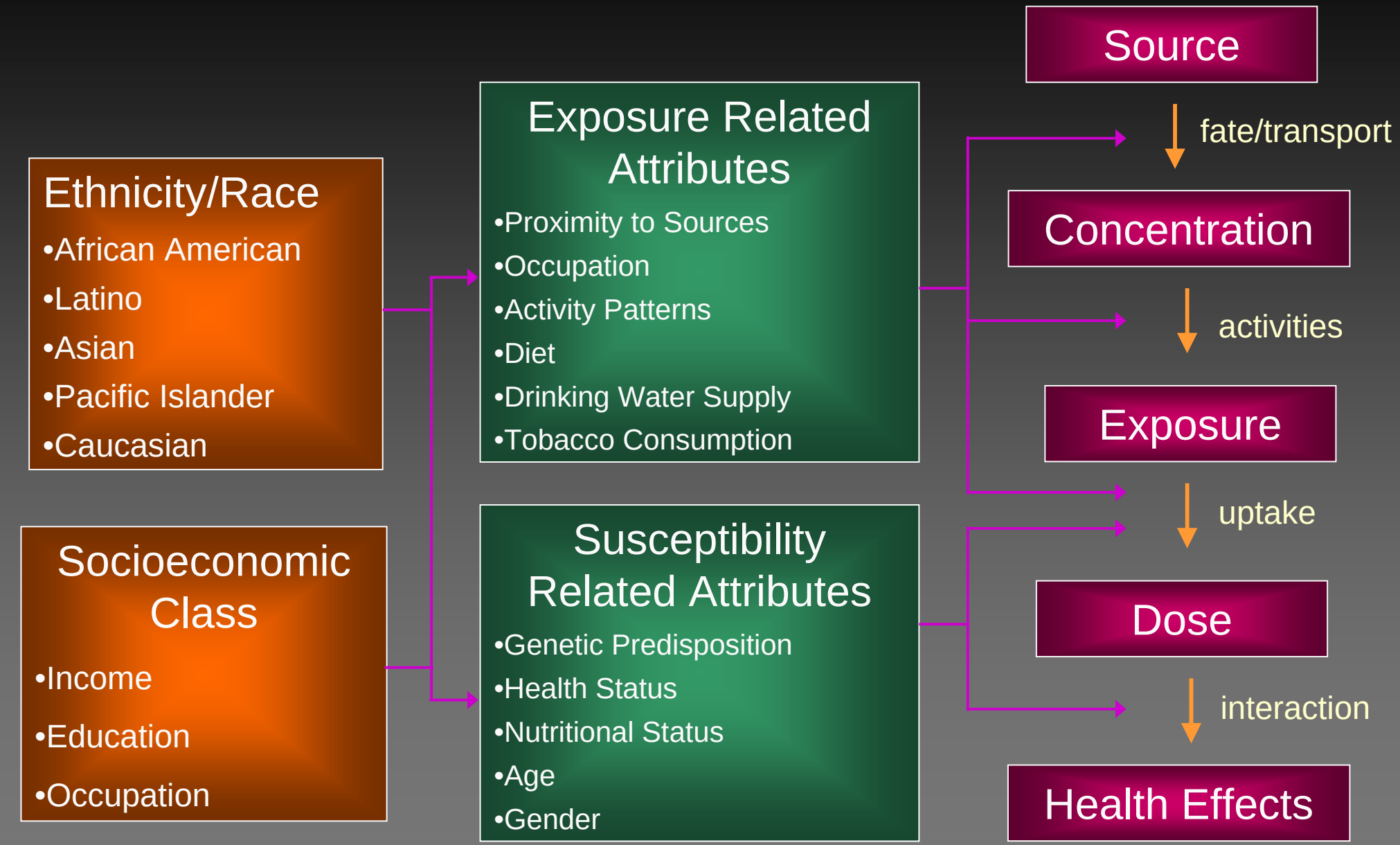
Although more expensive computational methods could be used, even the very inexpensive methods correlate well with higher level methods.



Recent computations for nanoparticles have shown differences in HOMO-LUMO energy gaps for different conformational structures, different metals or organics attached to those structures, and different sizes. This indicates HOMO-LUMO energy gaps for nanoparticles may correlate with toxicity like the other classes of compounds.

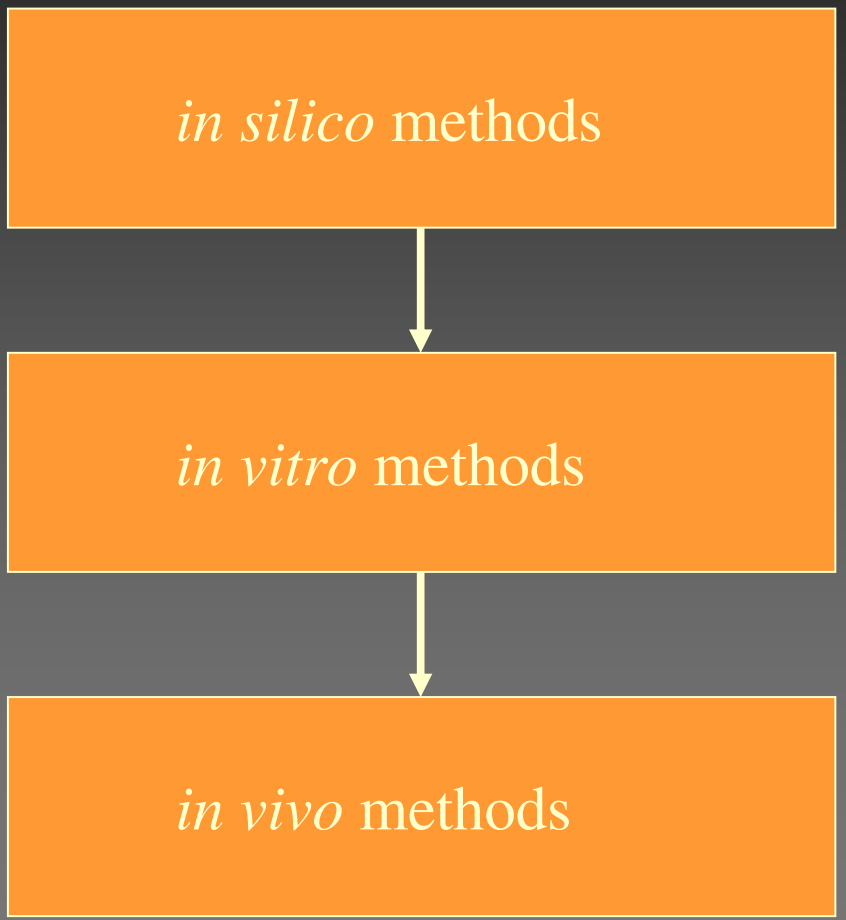
Strategy for determining risk



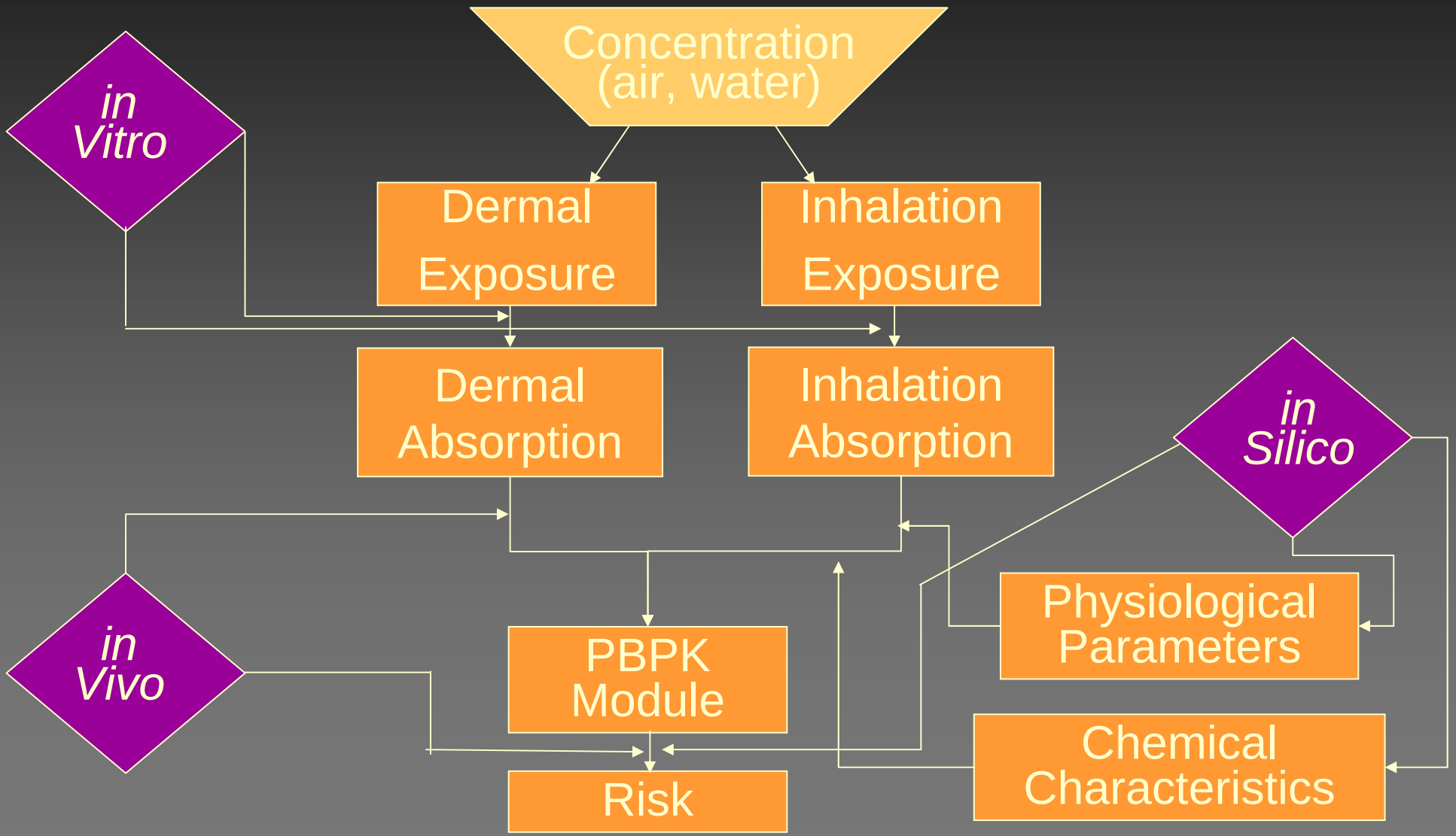


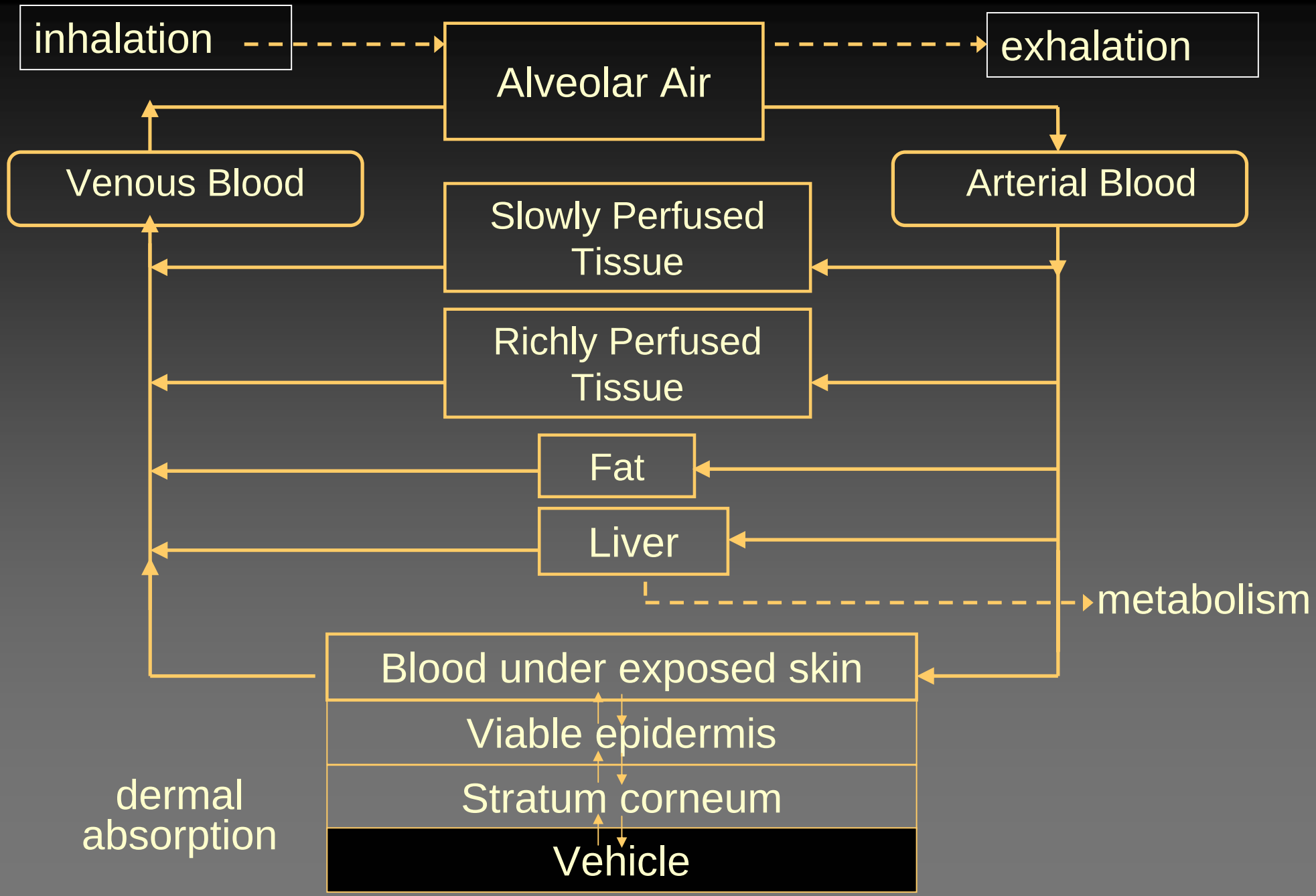
Adapted from Sexton and Adgate, 1999

Hierarchical Risk Assessment

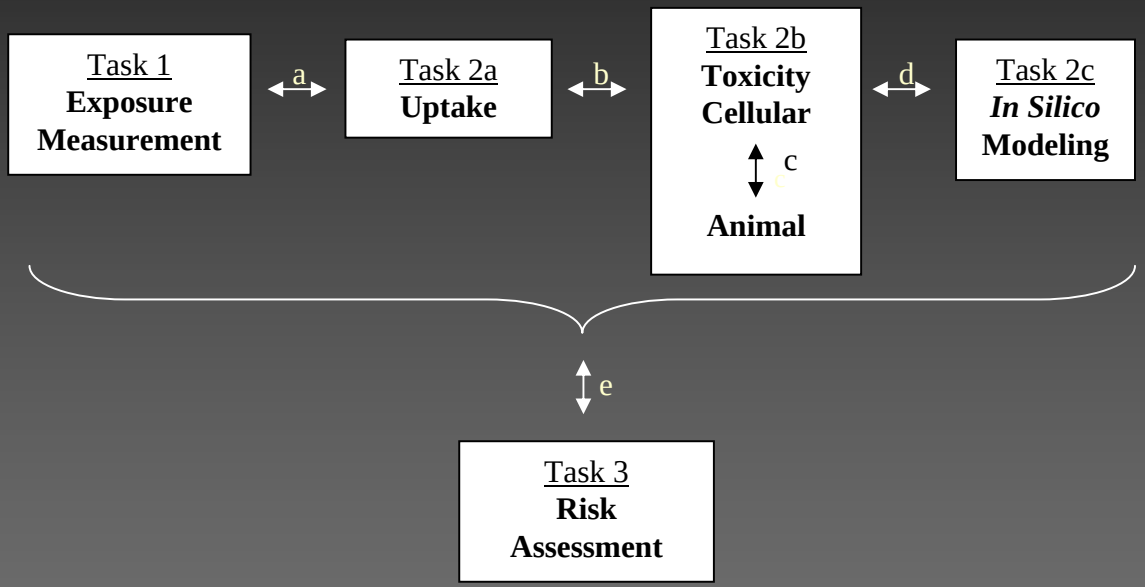


Risk Model Framework




inhalation
Alveolar Air
exhalation
Venous Blood
Arterial Blood
Slowly Perfused Tissue
Richly Perfused Tissue
Fat
Liver
metabolism
Blood under exposed skin
Viable epidermis
Stratum corneum
Vehicle
dermal absorption

Strategy for determining risk



Outcomes

- Development of methods to measure NPs
- Characterize uptake in skin and lung
- Assess toxicity both in vitro and in vivo
- Identify protein biomarkers
- Correlate QSAR with toxicity
- Develop a risk assessment model