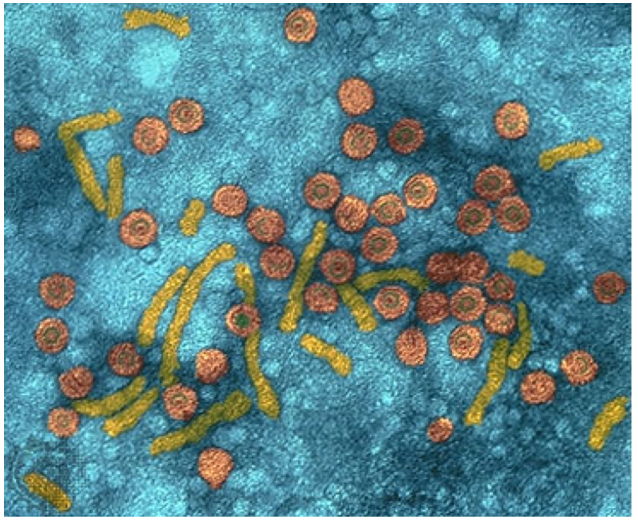

nano-Ag: PBTK Models, V_{blood} & NIOSH

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Purpose: Nanoinformatics & Computational Models

1. Examine a nano-Ag^0 computational model used in a regulatory context
2. SCCS has a nano-silver opinion
3. NIOSH time period overlaps with the nanoinformatics roadmap
4. NIOSH a case study for roadmap's dissolution pilot project
5. Roadmap will not be successful if it does not lead to predictive models accepted by regulators

NIOSH opinion & Bachler model utilize 'imperfect' information; do they inform each other?

Braque: Guggenheim

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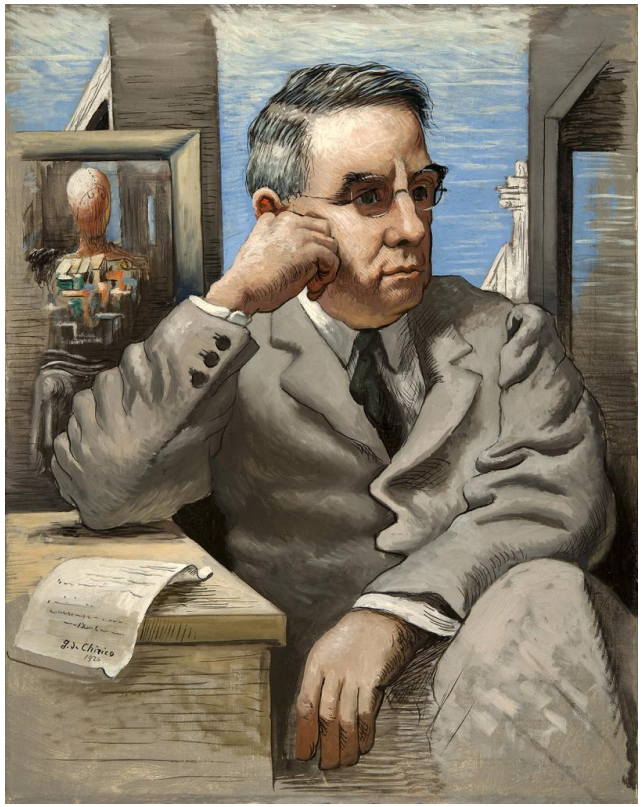
Argyrol, Argyria & nano-Ag

- 1902: Albert C. Barnes introduced a colloidal form of silver as a medicine;
- 1935: study of side effects in syphilitics & primary one is argyria, a skin discoloration;
- 1939: review for drinking purposes;
- 1954 biocide registered by EPA-predecessor;
- 1980 ambient water quality standard (EPA);
- 1990's drug use halted, remaining uses are in wound care, needless connectors (needle-less meant) and endotracheal tubes; and
- Worker exposure limits have been based on a mix of argyria and toxicity endpoints.



Barnes by de Chirico

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Living Proof

Argyria and silver toxicity respond to the same chemical factors with one difference being in photochemistry

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Roadmap Findings

1. Informatics:
   a) Deconstruct laboratory studies…. ***to***
   b) Populate databases…. ***in order to***
   c) Identify patterns & computational models to reconstruct reality, i.e. predict toxicity… ***in order to***
   d) Maximize knowledge & limit animal testing
2. Gaining regulatory feedback on models essential
   a) Done with drugs & less visible with industrial chemicals
   b) QSPR acceptance likely to occur before QSAR acceptance
3. Dissolution of sparingly soluble particles:
   a) Critical with QSARs & PBPK if both particle and dissolution product exhibit adverse effects
   b) Pilot project useful to identifying themes and actors

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NIOSH Draft

- 2016 Draft remained with 1988 & 2007 workplace REL of 10 µg/m^3 by inhalation
- 2018 Draft proposes 0.9 µg/m^3 for nano-Ag^0
- NIOSH draft:
  - mentions argyria frequently as observed in workers
  - no position taken tying argyria to toxicity;
  - each treated as independent manifestation of silver exposure
- NIOSH draft: no position (statement, review) of Bachler model's acceptability/credibility/relevance
  - Bachler *et al.* model used to relate particle size & concentration to argyria onset and relate the NOAEL to onset of pulmonary inflammation and bile duct hyperplasia

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PBTK-Thermo-Dissolution

- PB uses compartments to model a living entity
- TK is ADME acting on a toxicant
  - Overall exposure becomes a localized organ dose
  - Can scale across exposures, species, times
  - Mix of kinetic and equilibrium concepts (K_{ow})
- Particles complicate & challenge:
  - Uncertain dose metric & K_{ow} does not apply
  - Dissolution products replenished by solid ($\frac{d\,(c)}{dt} = 0$)
  - Handling adsorption (vascular system & protein corona) is an open question

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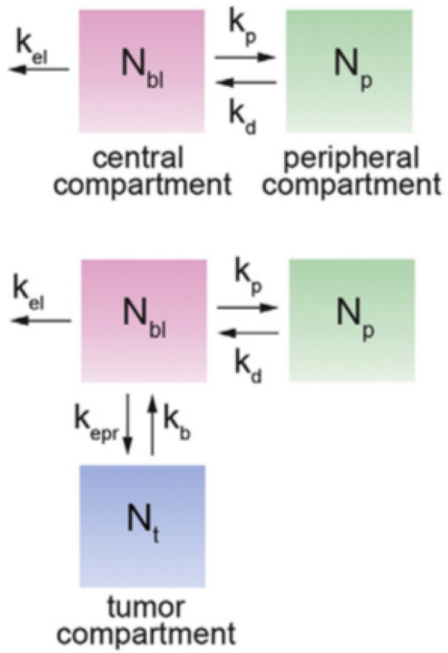
2 or 3 Compartments What's Missing?



- Problem: Extravasion critical factor with tumors
- PBPK challenge: tumor accumulation bundled into rate constants for clearance (kidney, MPS, etc.)
- Without 3 compartments miss:
  - Tumor-specific vasculature leading to different rate constants
  - Intravasation and elimination rates important for doxcorubicin effectiveness
  - For nano, different tools to balance extra- & intravasation rates for optimum delivery
- 3rd compartment allows for purposeful drug design strategies
- Purpose leads to # of compartments

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Loeschner & Bachler

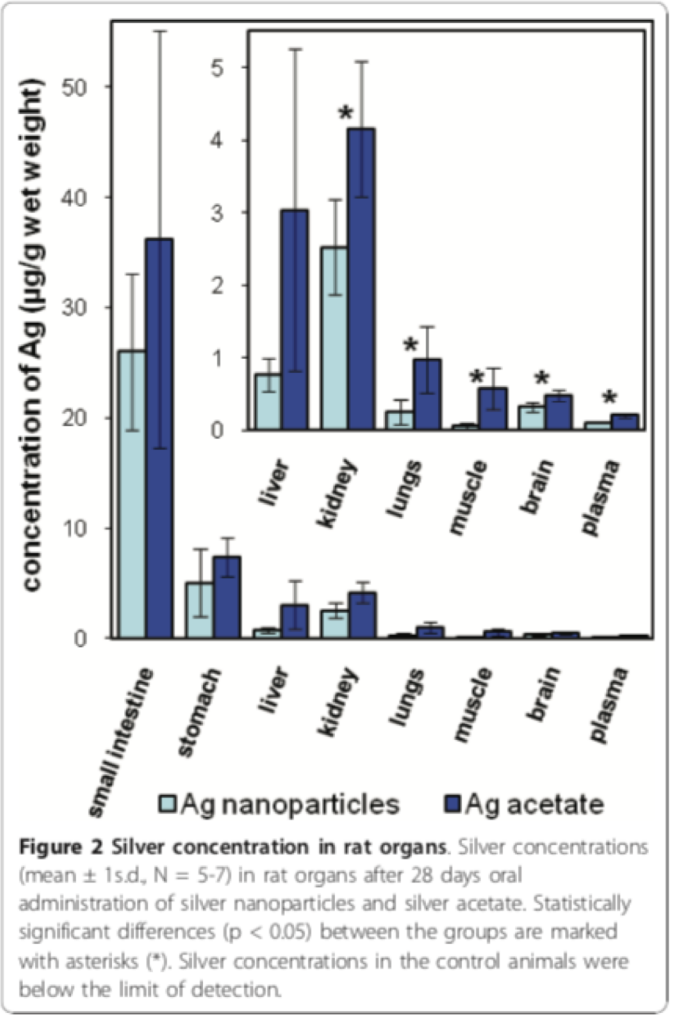
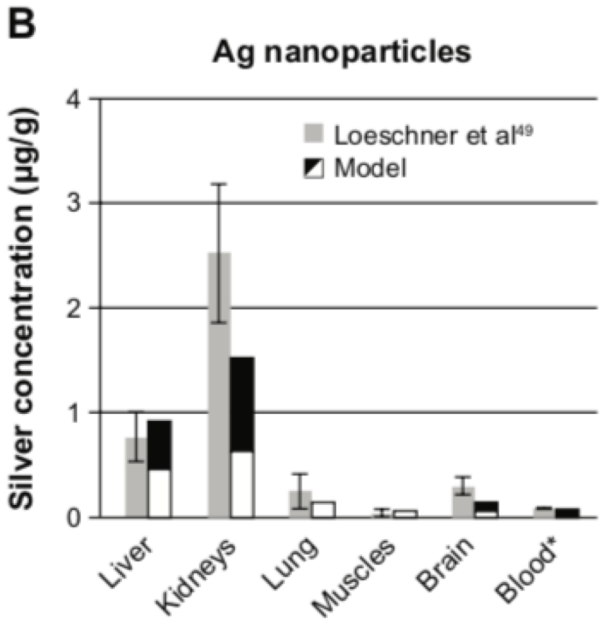


Figure 2 Silver concentration in rat organs. Silver concentrations (mean ± 1s.d., N = 5-7) in rat organs after 28 days oral administration of silver nanoparticles and silver acetate. Statistically significant differences ($p < 0.05$) between the groups are marked with asterisks (*). Silver concentrations in the control animals were below the limit of detection.



- Data used by Bachler are from inset;
- ~85% of Loeschner Ag not included;
- 'non-recovered' particles ascribed to bone marrow

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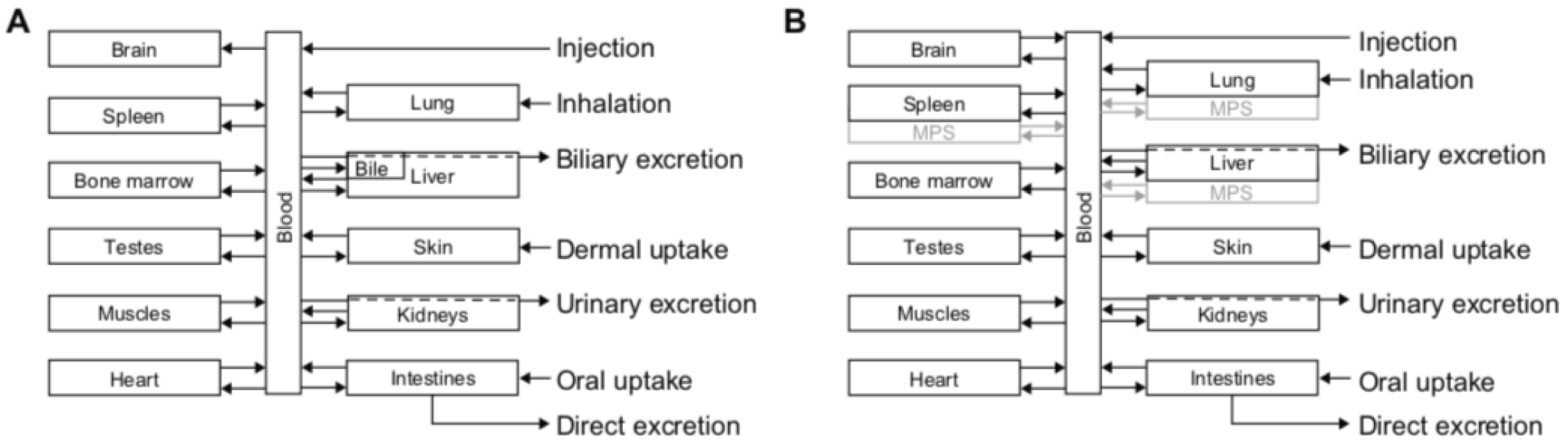
Bachler Physiology



1. No stomach (absent in gold inhalation; present in TiO2 food additive)
2. Bone marrow present, but not measured by Loeschner or Lankveld
3. Configuration is parallel pipe, not series
4. Other models would distinguish arterial from venous blood

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V_{blood} & Plumbing

$$k_{organ\_up\_ionic} = b_{ionic} * \frac{m_{organ} * c_{organ\_GSH}}{m_{b.w.} * c_{body\_GSH}} \qquad k_{organ\_up\_nano\_cap} = b_{nano\_cap} * \frac{Q_{organ\_blood}}{V_{blood}}$$

- Ionic silver:
  - Eqn. is organ ***mass*** & glutathione concentration
  - the term b_{ionic} is $[min]^{-1}$
  - calculates a pseudo-partition function for ***body***
- Particulate silver:
  - Equation is ***volume***, not mass, & ***blood***, not body
  - The term b_{nano_cap} is dimensionless
  - V_{blood} is series pipe configuration, not parallel
  - Distributes particles by organ blood flow rate

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Bachler & Argyria

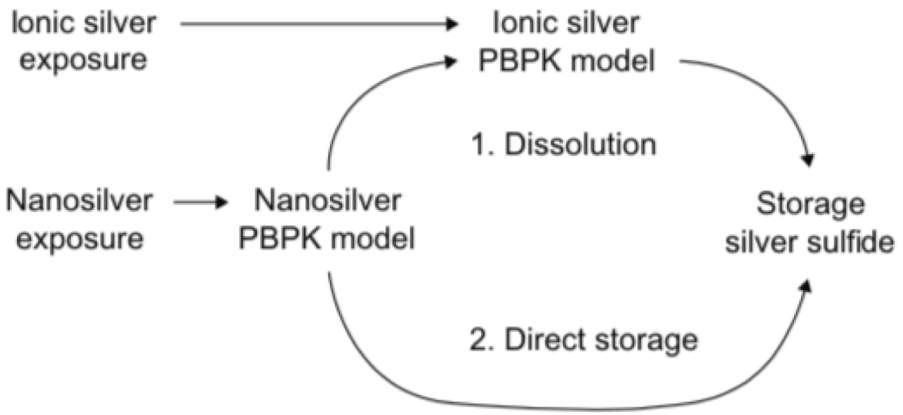

Figure 3 Metabolism of ionic silver and nanosilver and the connection between the PBPK models. For the fate of silver nanoparticles two scenarios were considered: (1) dissolution, and (2) direct storage as silver sulfide.
Abbreviation: PBPK, physiologically based pharmacokinetic.

- In diagram, Ag-ion and Ag-particle treated as independent;
- Argyria explanation (from Danscher) involves insoluble AgCl or Ag-phosphate as catalysts for photo-reduction to Ag^0;
- Argyria 'calibrated' using 1934 data as a threshold without relating to a mechanism through kinetic equations

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Particle Formation Absent

- Physiological (secondary) particles do form
  - Human stomach & skin, plants, MB, worms
- Hurt & Kane *et al.*: Ag^{+1}-glutathione ligand → Ag^{0} due to UV radiation
- Bigioni *et al.*:
  - Soluble metal 'particles' are discontinuous < 1 nm
  - Present as 'magic number' clusters (8, 20, 40, 58..)
  - Distribution responds to glutathione or Ag^{+1} conc.
- Concentration gradient driving force of *in vivo* dissolution offset by photoreduction

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Bachler Model & Dissolution

- "best agreement" of model & data occurs with "no dissolution of silver nanoparticles"
- Ag^{+1} ions & Ag^{0} particles follow separate paths
  - Stomach, where particles & ions mix, used to estimate intestinal exposure, but not part of model
  - Prefer intracellular dissolution to explain argyria
- Known phenomenon, not model, discounted
  - Daphnia medium: 9 µg/L → 88 (7 d) → 146 (28 d)
  - Algae medium: 18 µg/L → 125 (7 d) → 214 (28 d)
  - Slow increases likely due to ligands forming

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NIOSH & Bachler: Fazit

- Both remain challenged by argyria
  - Visible, known, historical phenomenon requiring explanation
  - Neither discusses sunlight
  - Neither offers mechanistic explanation beyond 1935 threshold
- Both address dissolution episodically
  - source of ions (toxicity) and particles (toxicity & argyria) primarily discussed as nano- *vs*. bulk before administration
  - underscores reliance on EHS over the P-Chem literature
- Bachler model states in mathematics what NIOSH states in words; neither informs or builds on the other
- NIOSH provides Bachler with an *imprimatur*

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EFSA Acceptance of GUTS for Plant Protection Products

1. Framework
   - Definitions, equations, 'accepted' interpretations
2. Implementation
   - Math package (Mathematica, R)
   - Two 'ring' data sets to verify new implementations
3. Selecting case study modules
   - based on experimental design & data
4. Regulator can validate with FOCUS scenarios
   - web accessible Excel implementation from CNRS

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Nano-PBTK Thoughts

1. Modeling practice resembles other fields with a similar range of usage & abusage and would benefit from 'informatics' rigor
2. Verification should entail:
    a) Physical plumbing (parallel or series) and include a 'remainder' compartment
    b) Replicating accepted results of small molecules
3. Validation should entail:
    a) Clarity on equilibrium, non-equilibrium and kinetic concepts
    b) P-chem assumptions should describe the calibration solution's composition
    c) Include dissolution + particle formation for nano
    d) Should recognize the inter-relationship with QSAR and QSPR descriptors
    e) Should consider the MIEs of AOPs

Braque: Guggenheim

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Thank You

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Citations

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