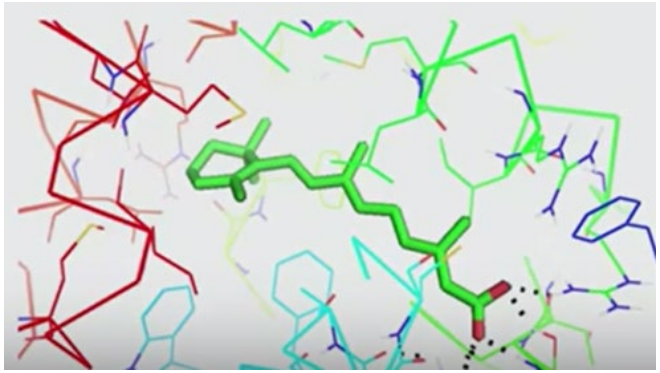

Toxicokinetics (Absorption, Distribution, Metabolism and Elimination) of Toxicants

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February 12, 2019

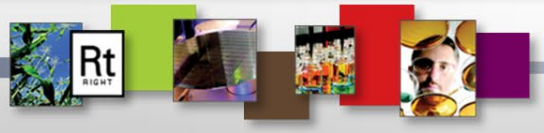
Agenda

- What is Toxicokinetics (TK)?
- Chemical Factors Favoring Absorption
- Understanding Toxicokinetics
- Toxicokinetics: Route Dependent
- Toxicokinetic Linearity vs. Nonlinearity
  - Case study: Saturated Absorption
  - Case study: Saturated Elimination
- *In Vitro* and *In Si*lico Approaches to Evaluate TK
- Conclusion

Toxicokinetics

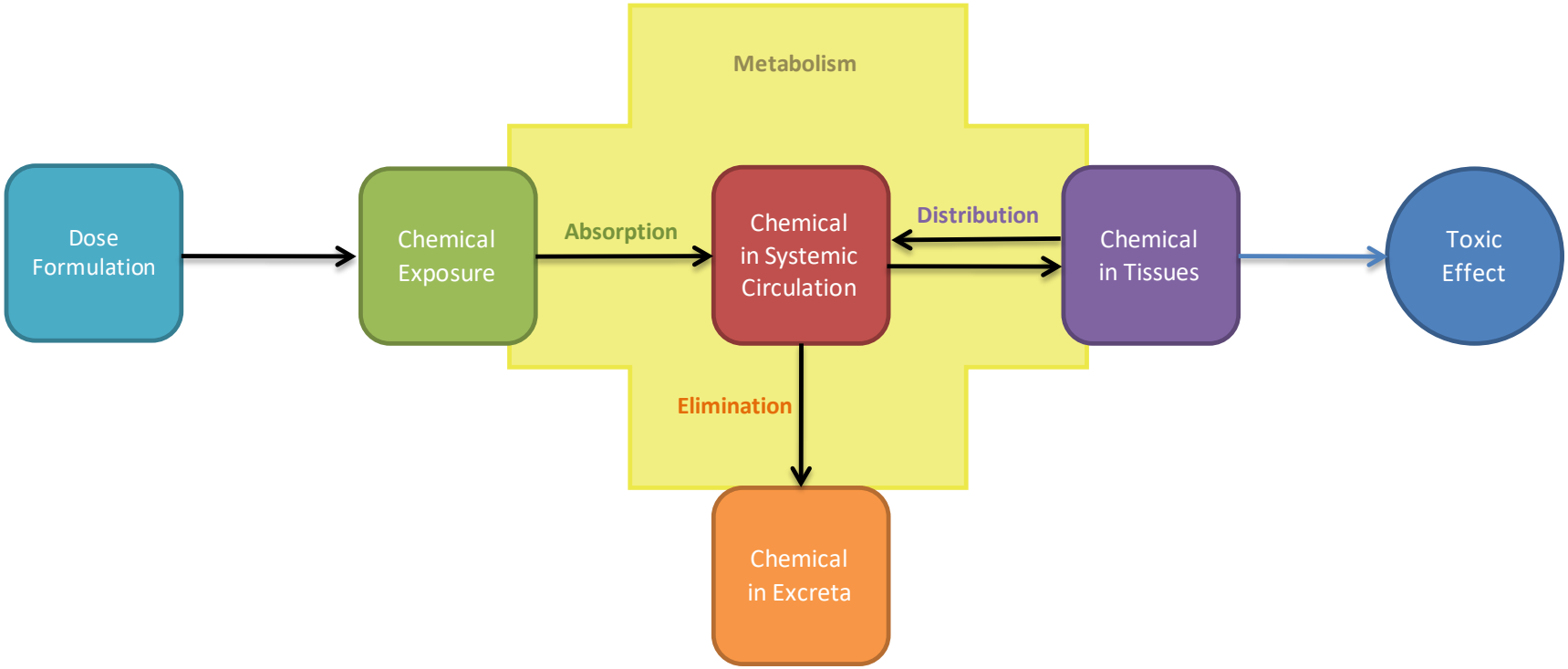
- Toxicity is related to exposure, toxicokinetics, and toxicodynamics

- Toxicokinetics: Effect of a hazardous substance depends on the level or concentration of the substance that is present in the body's systems – this will depend on rates (or kinetics) of
  - **A**bsorption – chemical uptake
  - **D**istribution – movement to tissues throughout the body
  - **M**etabolism – biotransformation of parent compound
  - **E**limination – removal from the body (urine, feces)
- Studying the internal exposure over time



What is “Toxicokinetics?”

The study of internal exposure over time.



Value of Toxicokinetic Data

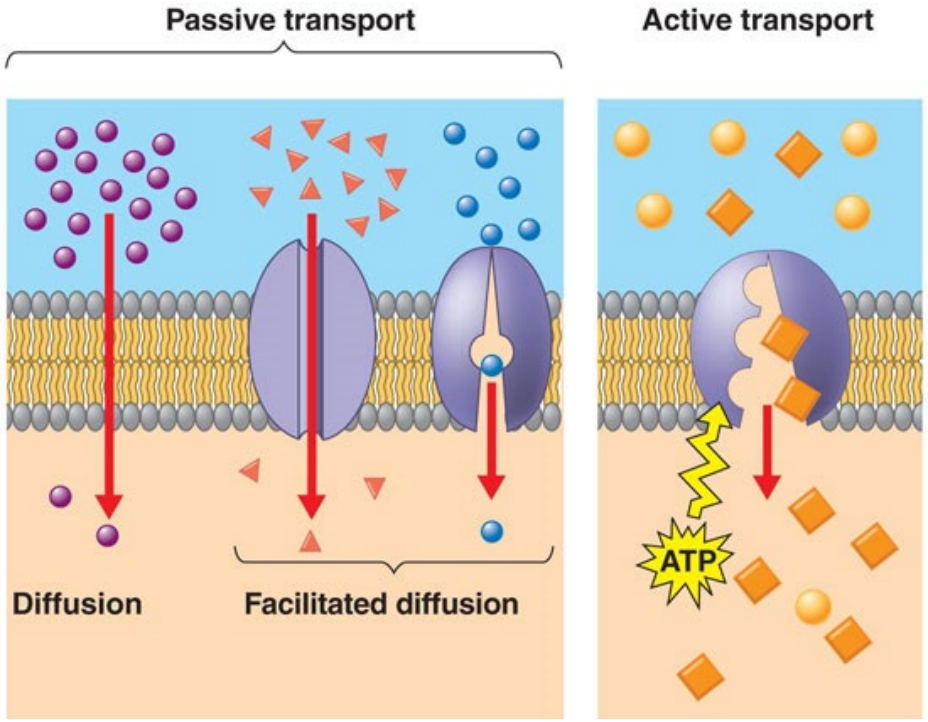
- TK Data Provide:
  - Understanding of systemic exposure in toxicity studies
    - Explain toxicity observations (correlate tissue dose with toxicity)
    - Identify proximate toxicant (parent or metabolite)
    - Understand route, species, gender or life-stage sensitivities to toxicant exposure
    - Provide relevance for toxicity data (linear vs. non-linear TK)
  - Evaluation of margin of safety in humans by:
    - Correlation of animal effects at known systemic exposure to measured human biomonitoring results

Chemical Factors Favoring Toxicant Absorption

- Lipophilicity (high log P, K_{ow})
- Small size
- Neutral charge

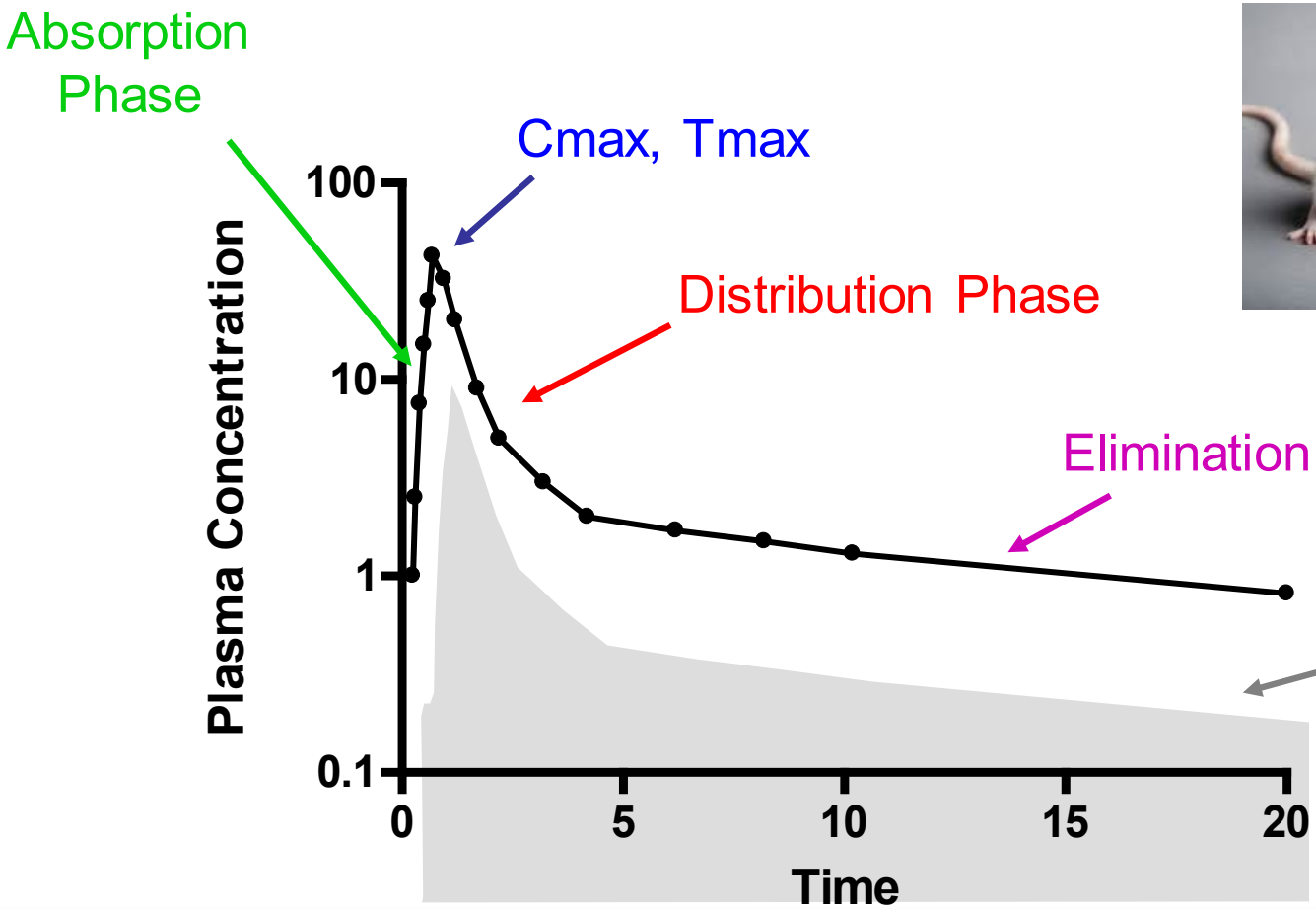
→ greater absorption

- Movement across membranes by diffusion, facilitated diffusion and active transport



Understanding Toxicokinetics

Typical pharmacokinetic time-course following oral administration



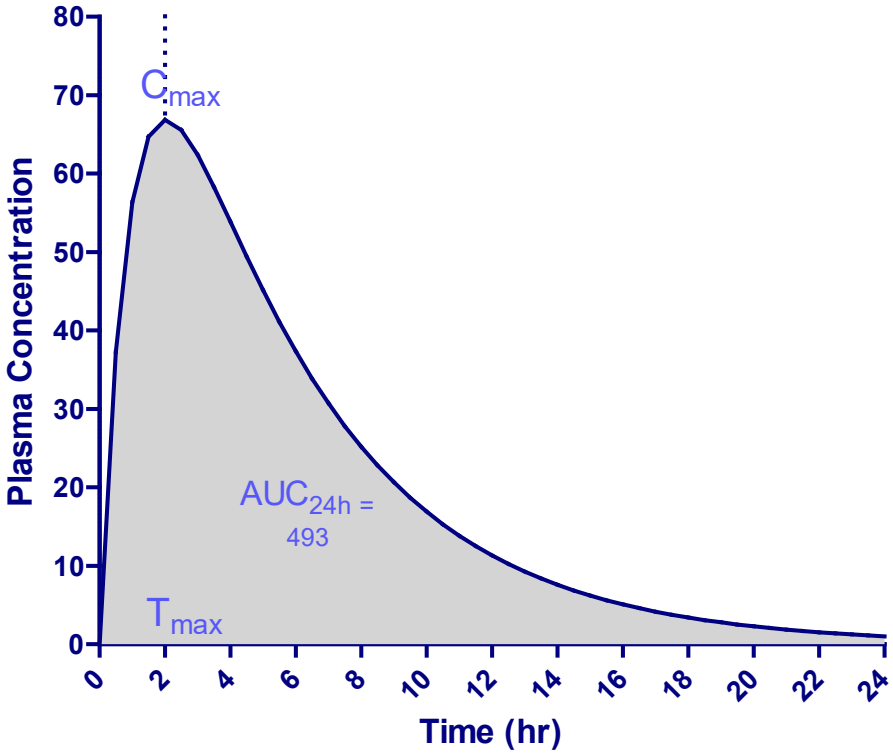
Understanding Toxicokinetics

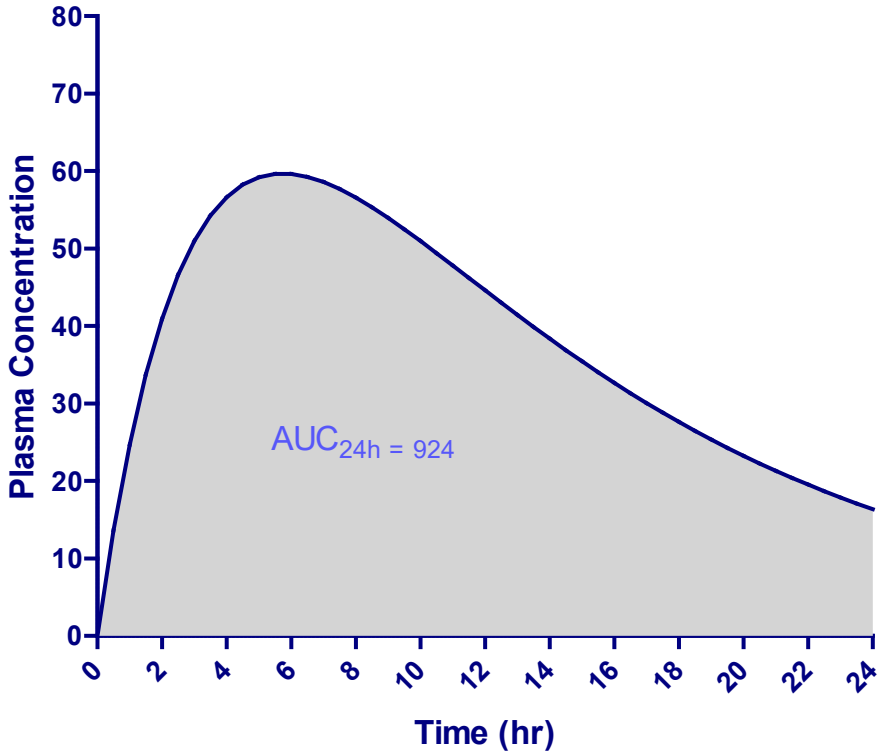
- C_{max}:
  - Maximum blood/plasma concentration
- T_{max}:
  - Time of Cmax
- AUC_{24h}:
  - Area under the plasma concentration-time curve from 0 – 24 hours
- Half Life ($t_{1/2}$):
  - Time at which half of the dose has been eliminated from the body

Understanding Toxicokinetics

The study of internal exposure over time.

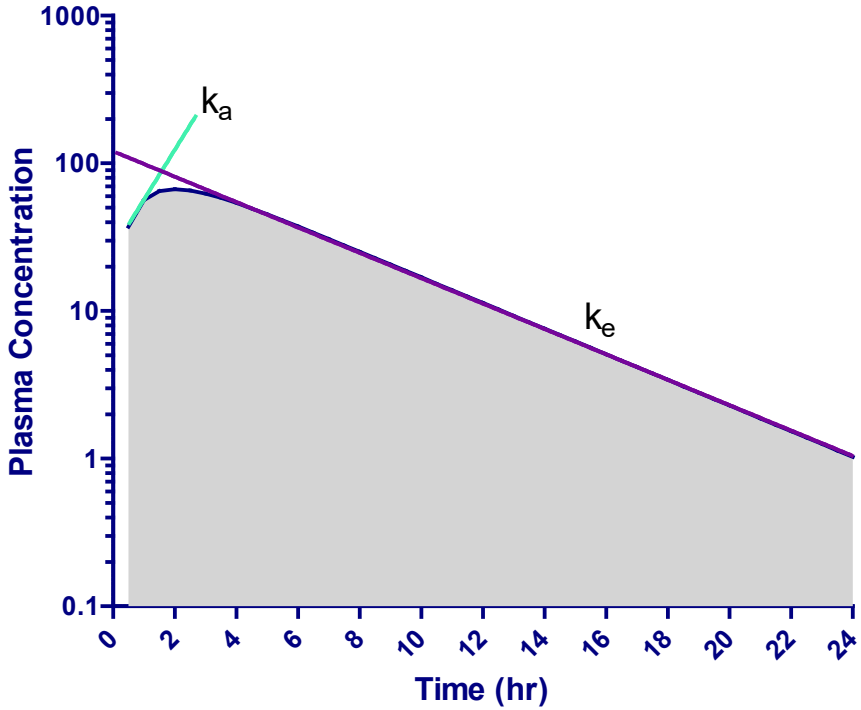
Cmax: Maximum Concentration and AUC: Area Under the Curve

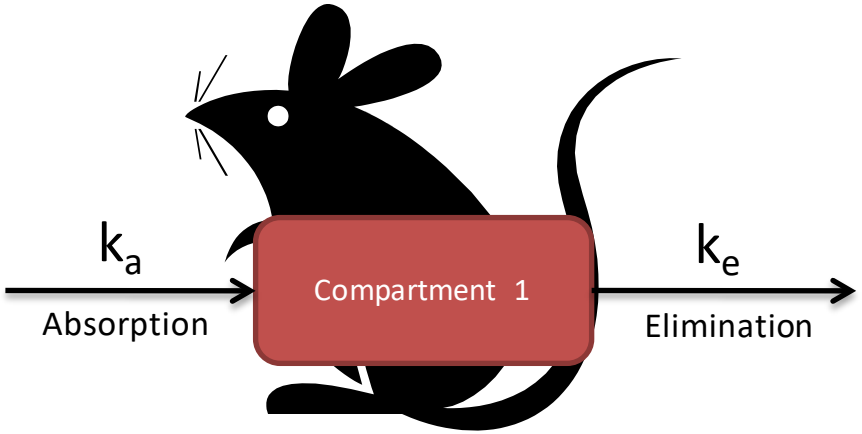




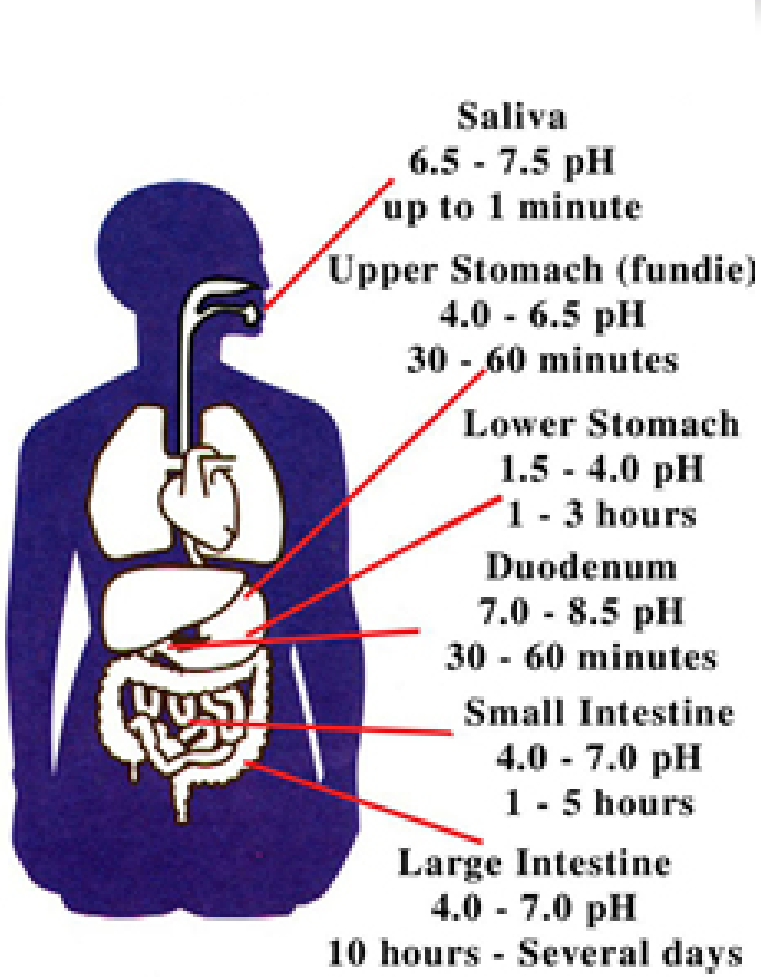
Understanding Toxicokinetics

Toxicity is dependent on absorption, distribution, metabolism and elimination





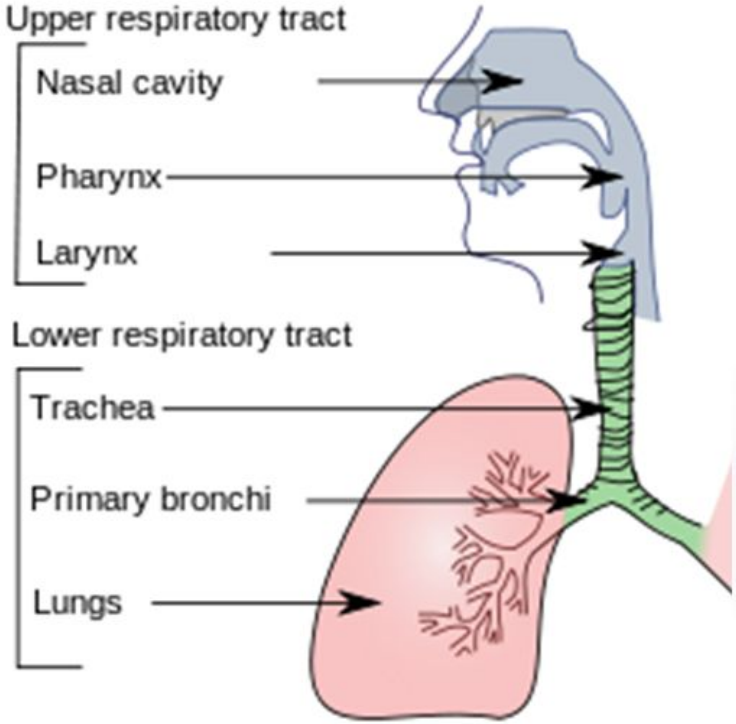
Toxicokinetics: Route Dependent - Oral

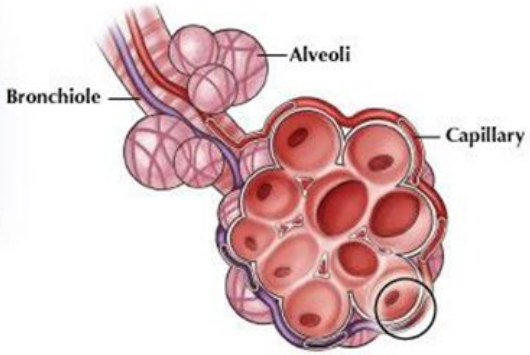


- Compounds can be absorbed throughout the GI tract depending on time spent in contact, surface area and pH.
- Weak acids more easily absorbed in stomach; weak bases in the gi tract (neutral charge)
- Blood from gi to portal vein to liver for biotransformation
  - Detoxification
  - Bioactivation
  - Elimination in bile

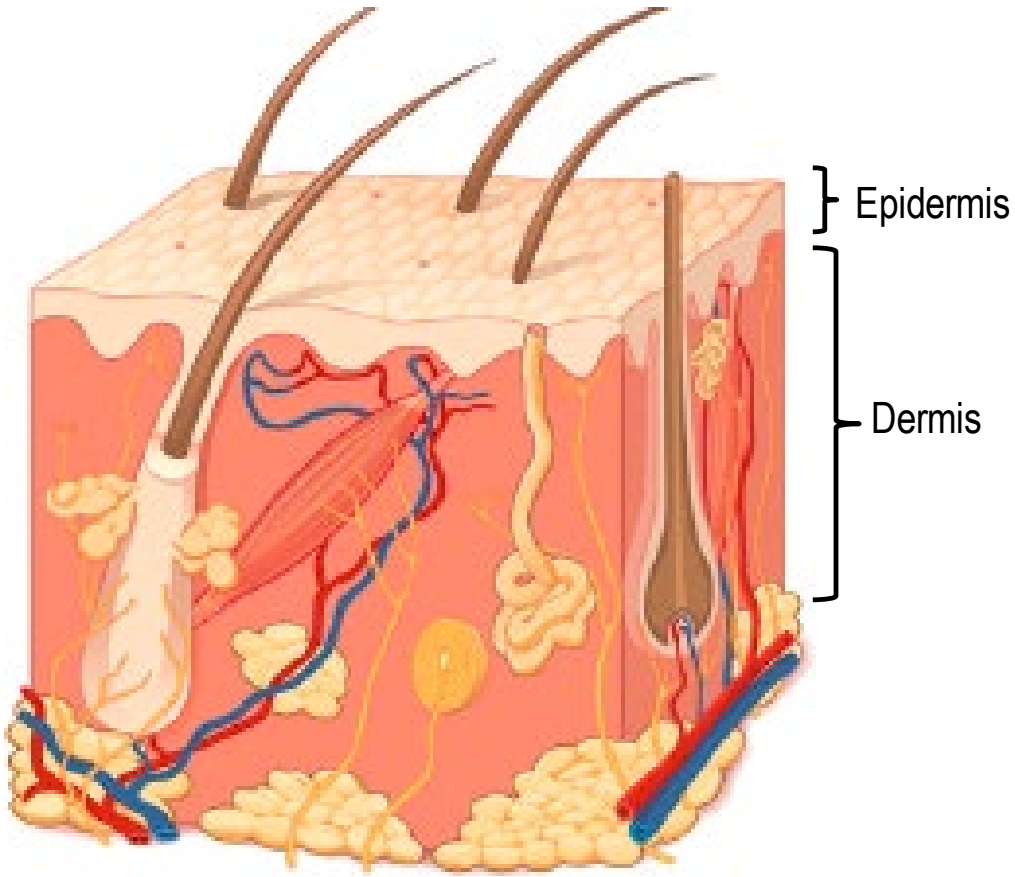
Toxicokinetics: Route Dependent - Respiratory

- Gases and aerosols (particles)
- Water soluble gases absorbed in upper respiratory tract; less water soluble can reach lungs
- Particle size determines deposition; 4 µm respirable fraction
- Compounds reaching lungs can be readily absorbed





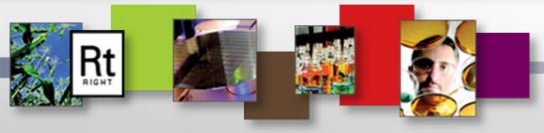
Toxicokinetics: Route Dependent - Dermal



- Skin has greatest cellular barrier to absorption (thickened cell membranes)
- Lipid soluble chemicals can penetrate into epidermis and dermis
- Species differences: Dermis is thinner in laboratory animals, but covered in hair; humans have greater blood flow for absorption

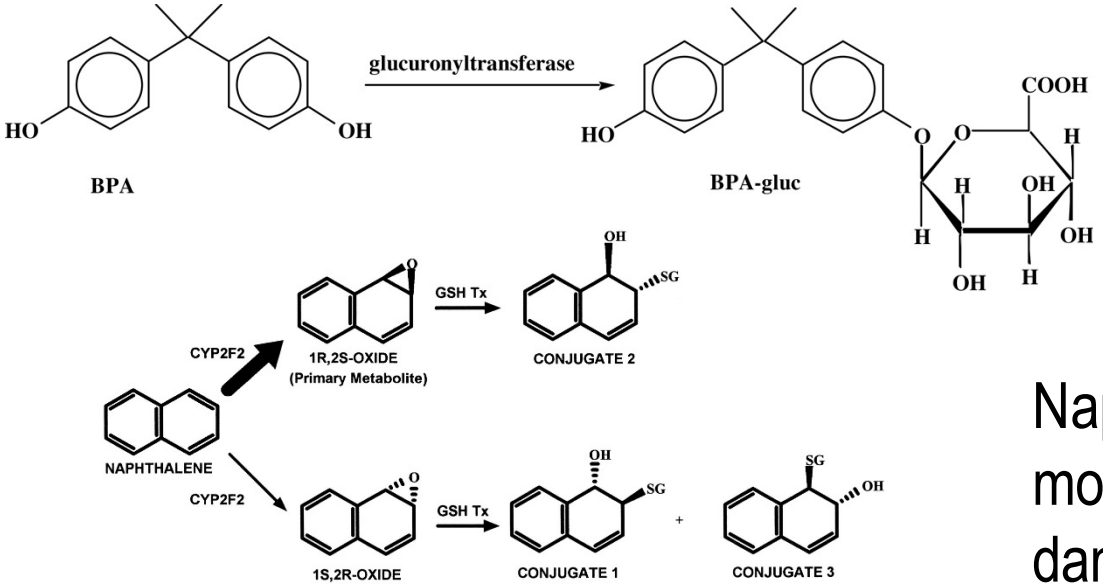
Toxicokinetics: Route of Exposure

- Route of exposure carries different concerns for absorption, bioavailability, metabolism, etc.; therefore, exposures in toxicology studies should use a relevant route of exposure



Metabolism: Detoxification or Bioactivation?

- Balance between bioactivation and inactivation can determine chemical toxicity to cells/tissues
  - Formation of a non-toxic metabolite
  - Generate a toxic metabolite which is then detoxified
  - Generate a toxic metabolite that results in cellular/tissue damage



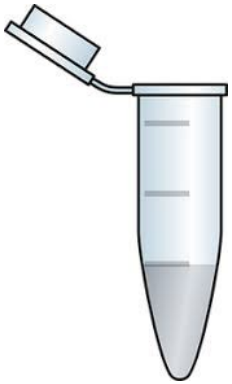
Bisphenol A is glucuronidated to an inactive metabolite.

Naphthalene forms epoxides in mouse lung, resulting in damage.

Toxicokinetics Subdisciplines

- We use various approaches to achieve our desired endpoints:

In vivo

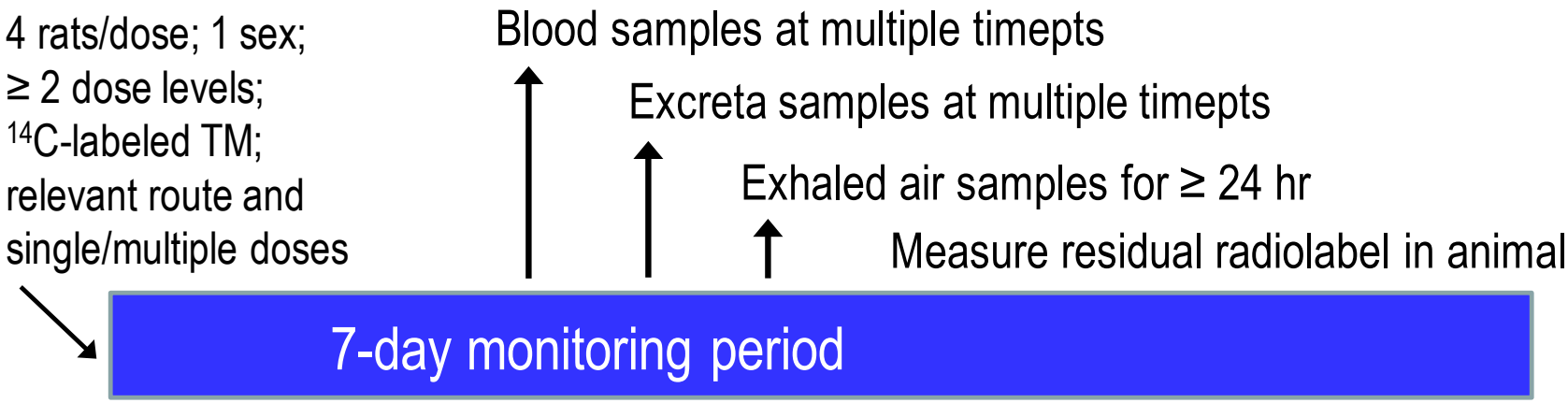


In vitro

In silico

In vivo Approaches to Examine TK

- OECD 417 test guideline on Toxicokinetics
  - Demonstrate systemic exposure, circulating moieties, potential for accumulation in tissues, biotransformation

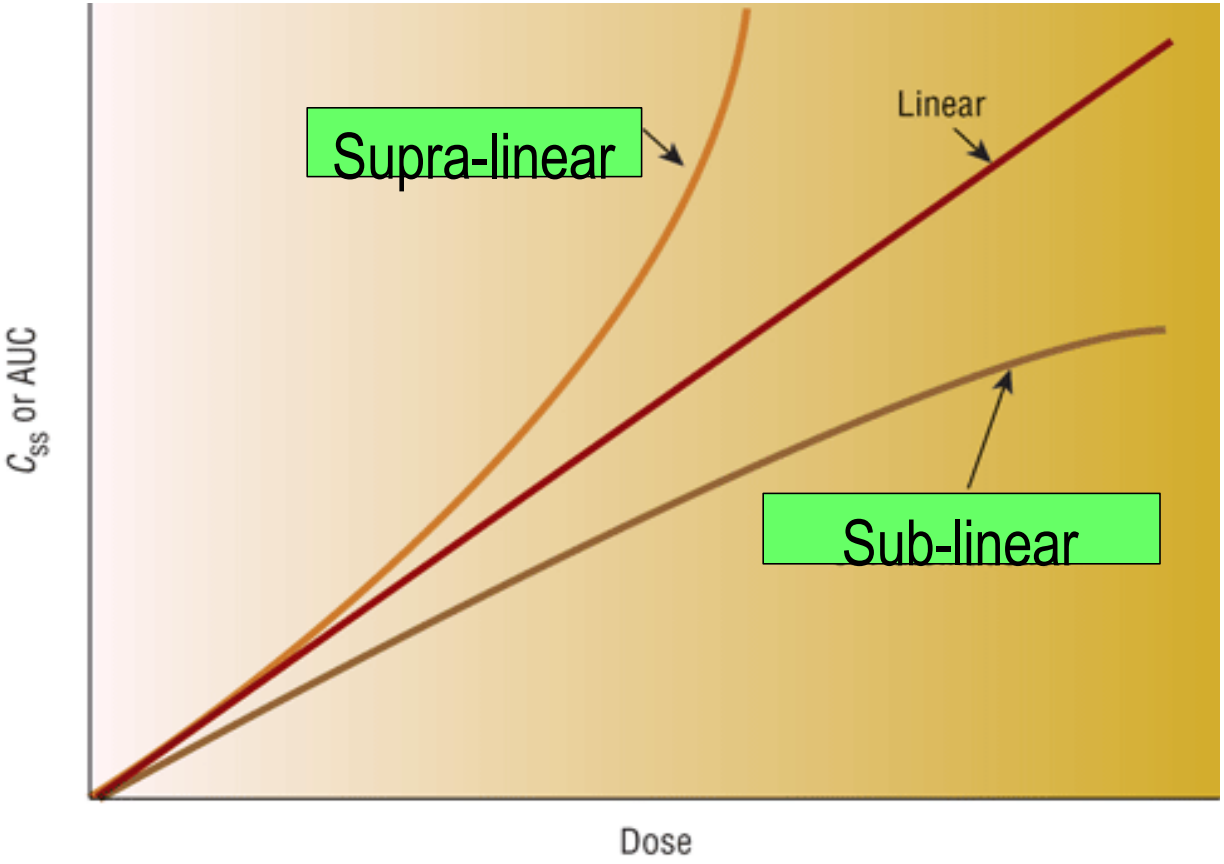


^{14}C-TM located in metabolically stable portion of the molecule:

- Identification of metabolites with adequate sensitivity
- 90% Mass balance required
- Collect blood at Tmax for metabolite identification

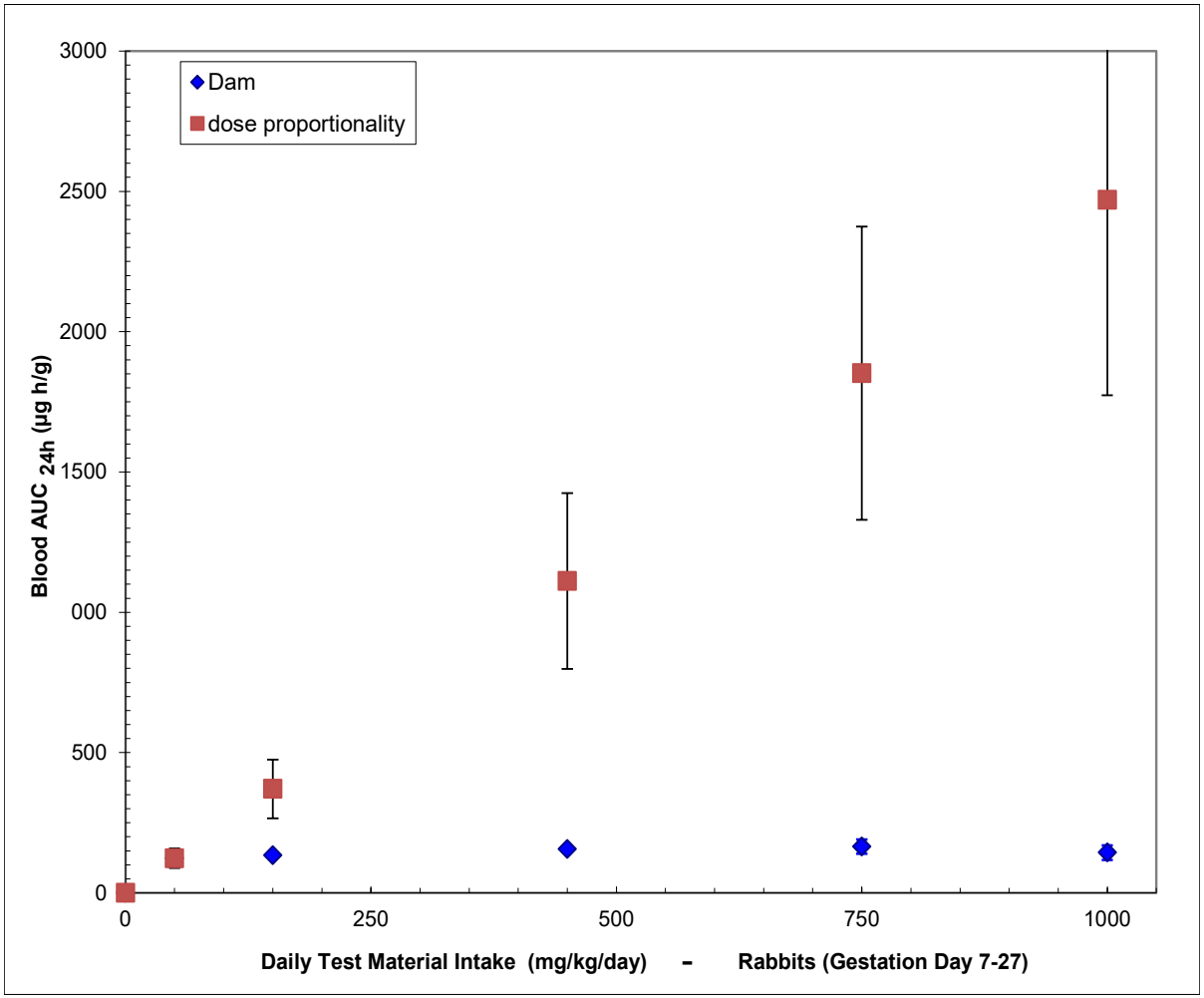
Toxicokinetic Linearity

Linear TK is when the internal dose increases proportionally with the applied dose



TK can be supra-linear or sub-linear (higher or lower internal dose than expected) if absorption, distribution, metabolism or elimination become saturated.

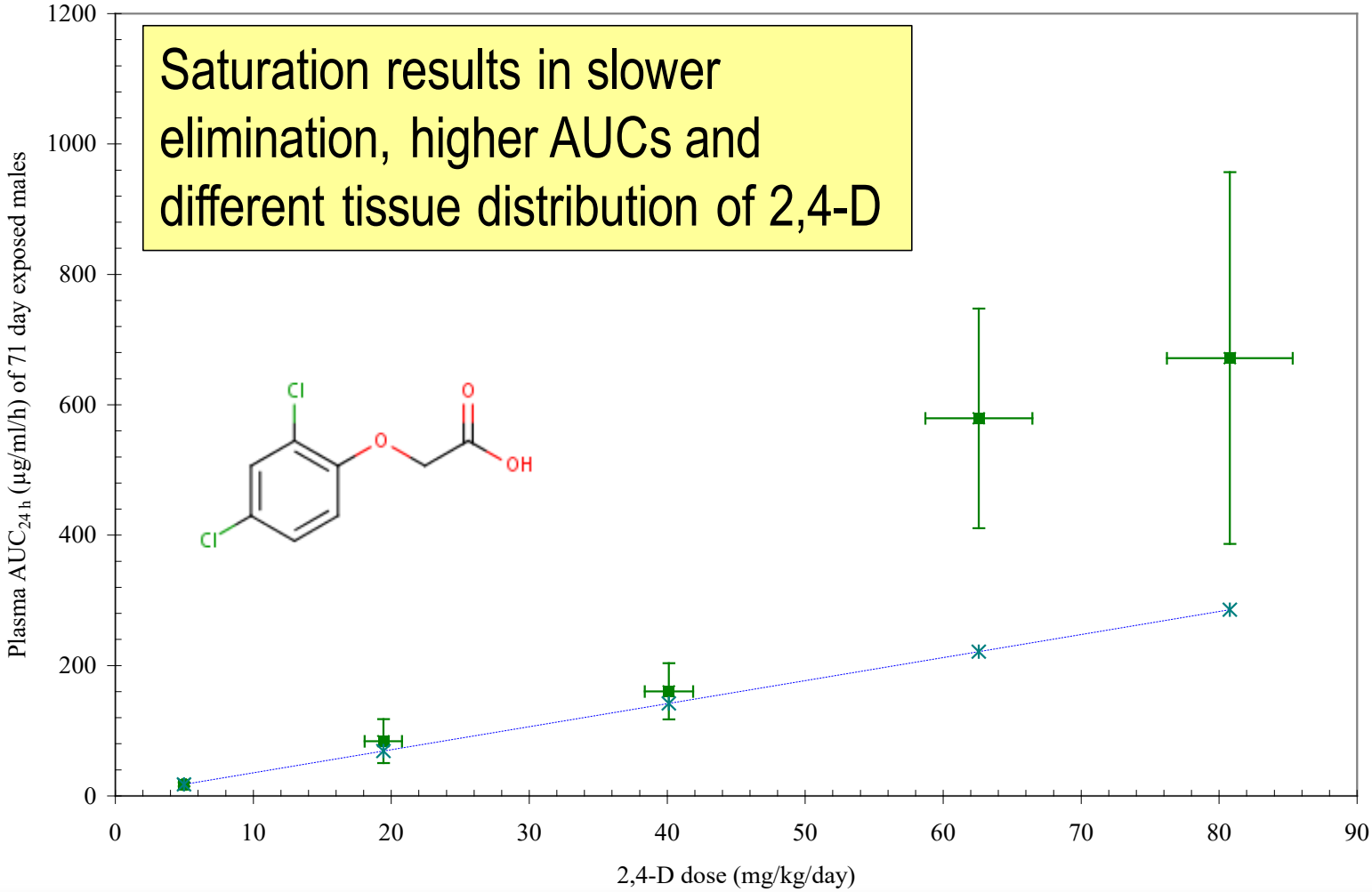
Case Study with Silicones: Saturated Absorption



- Higher exposures ≠ higher internal dose
- Toxicity may not exhibit dose-response relationships

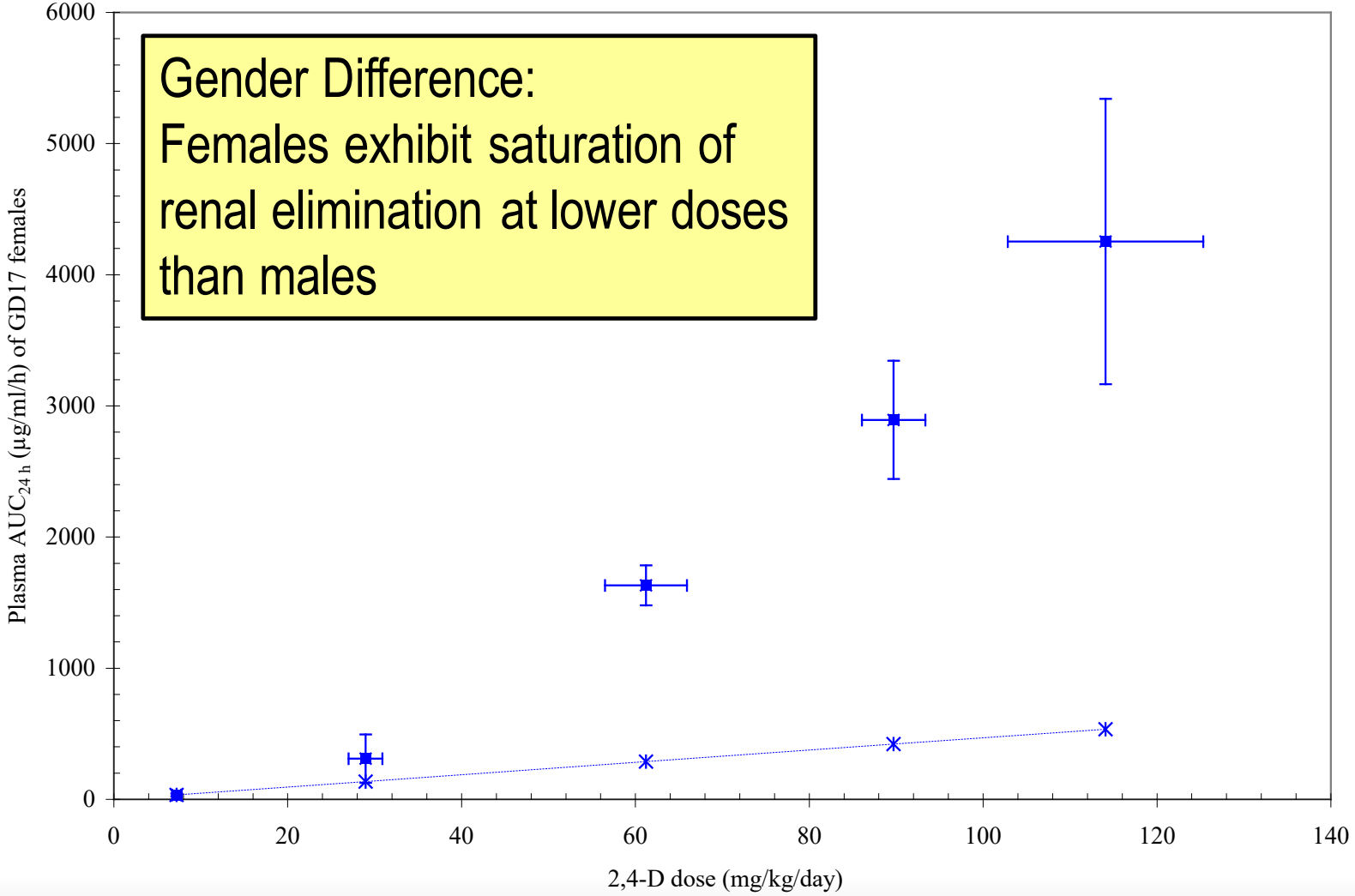
2,4-D Case Study: AUC is not Dose-Proportional in Male Rats

(blue line represents the expected dose proportional increase based on the low dose value)

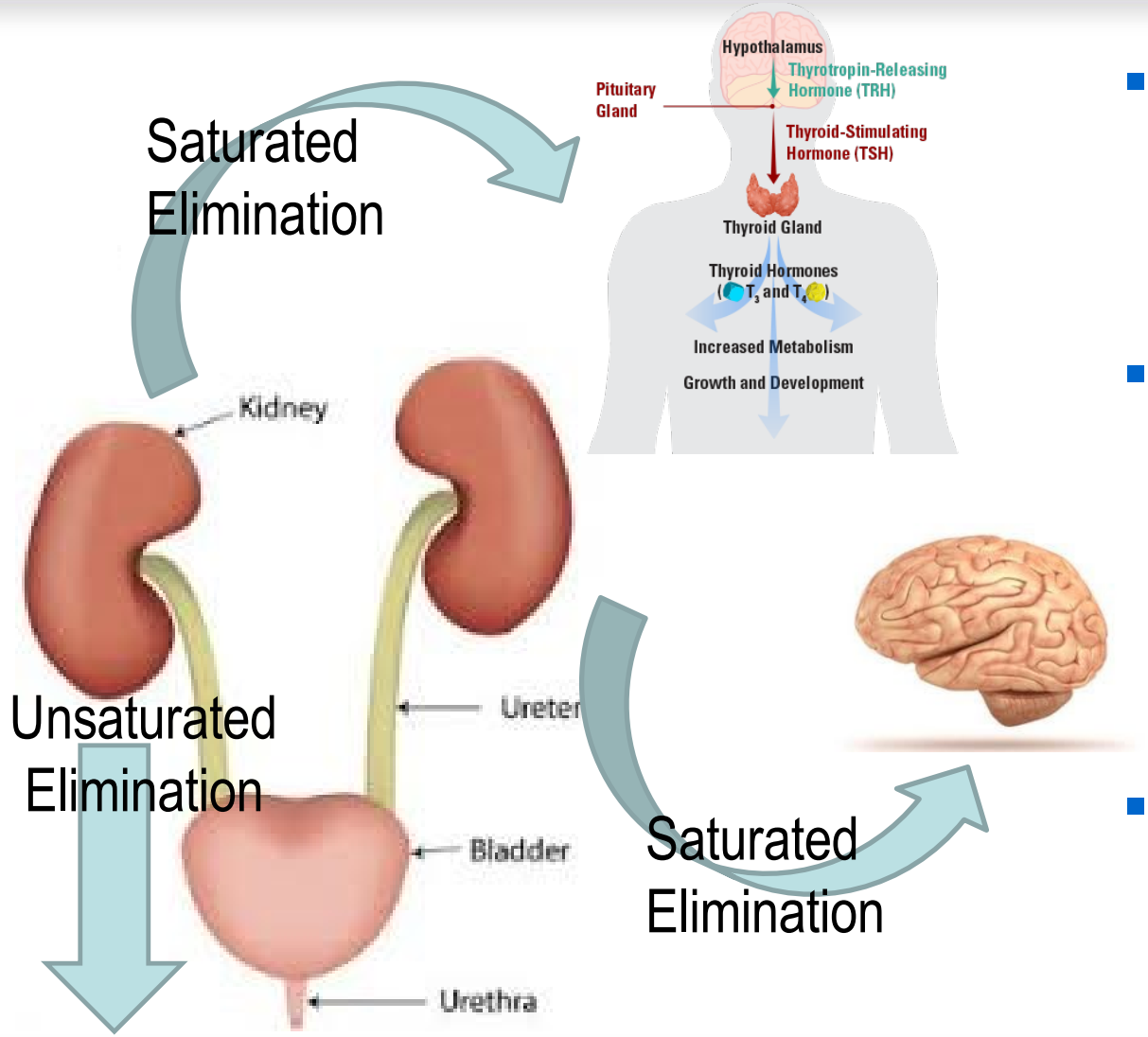


2,4-D Case Study: AUC is not Dose-Proportional in Female Rats

(blue line represents the expected dose proportional increase based on the low dose value)

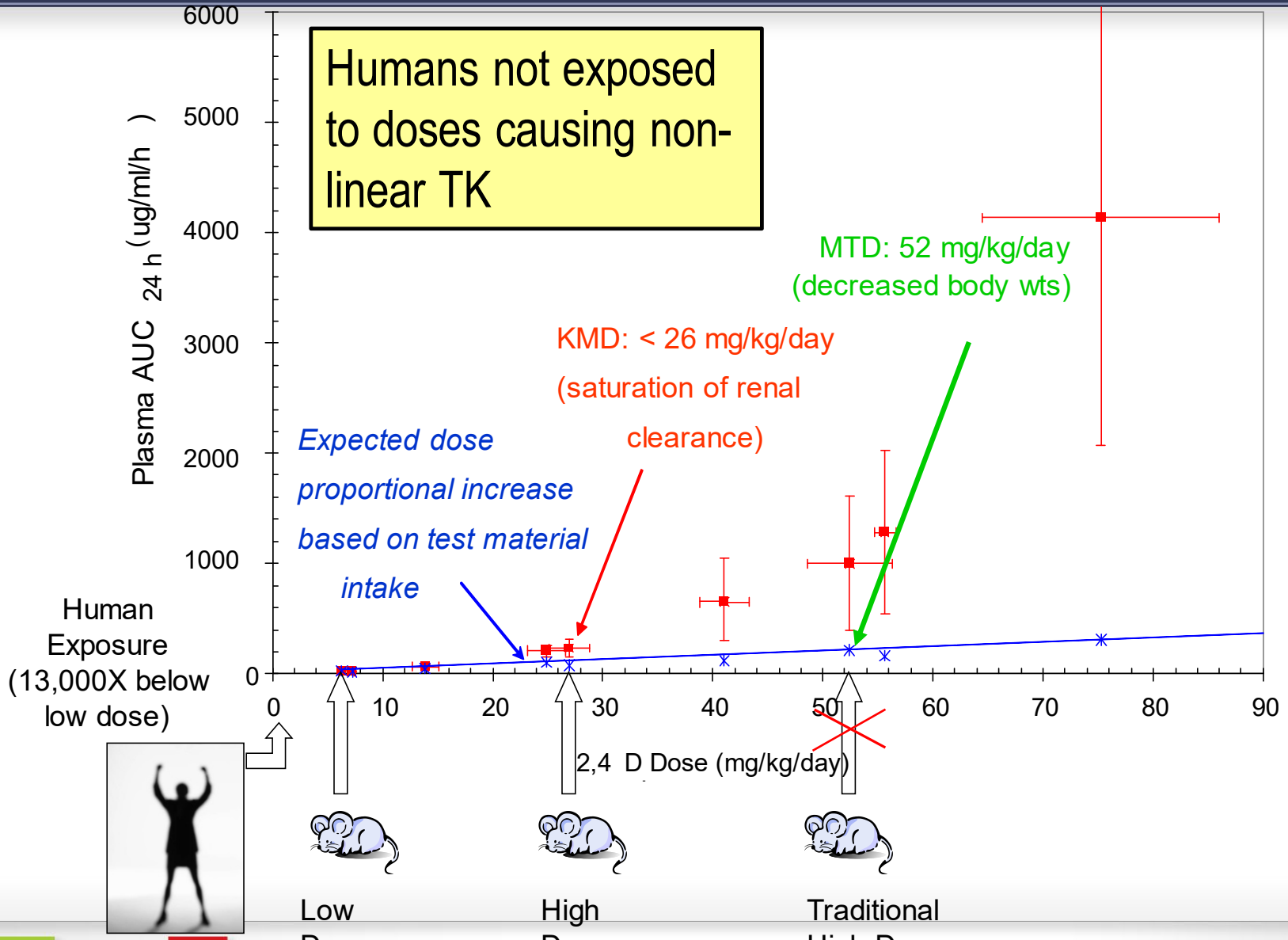


2,4-D Tissue Distribution with Nonlinear TK



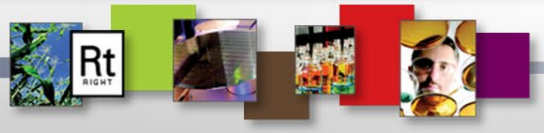
- Human exposures are lower than animal studies (transporter not saturated)
- With saturated elimination (non-linear TK), 2,4-D can be redistributed to affect thyroid hormone transport and the brain.
- These targets are not affected at lower doses (linear TK range)

Kinetically Derived Maximum Dose (KMD) Determination

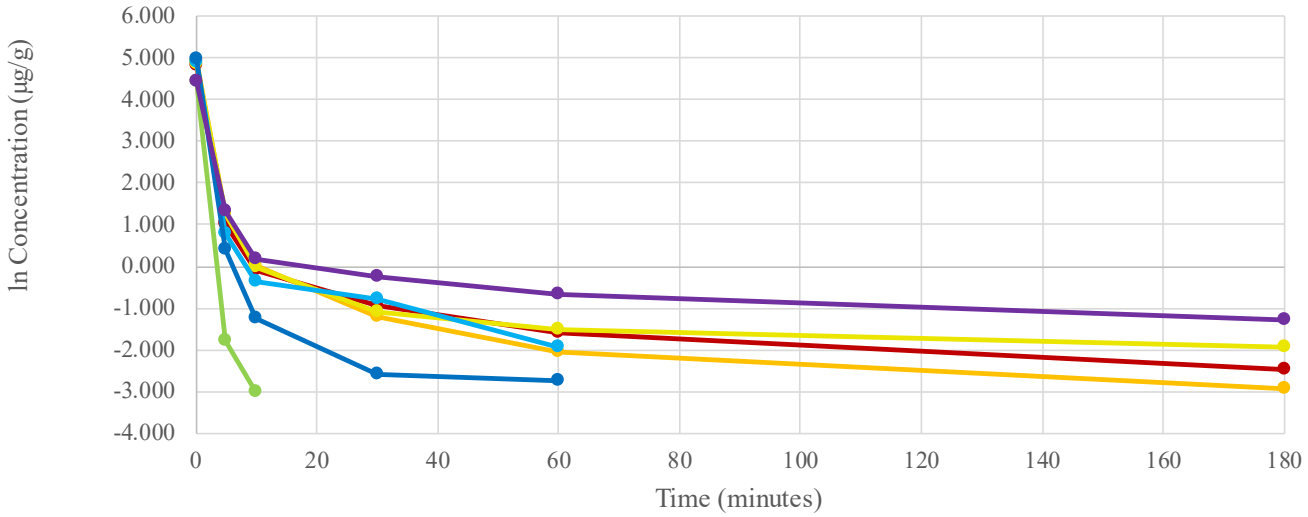


Toxicokinetics Parameters

- Toxicokinetics can differ due to:
  - Species
  - Gender
  - Age/life-stage
  - Dose and dose rate
- Important questions when examining toxicology study data:
  - What are dose levels? What are human exposure levels?



in vivo Application: Hydrolysis Reactions for Read-Across



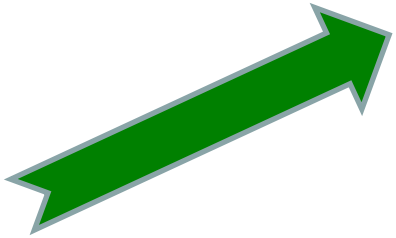
Filling gaps in toxicity testing to minimize animal use

'Read Across' for Related Chemistries:

- Read across by bioactivity or TK
- Can test the two extreme cases and interpolate other results

Predictive Toxicokinetics

Using *in vitro* or *in silico* models to predict absorption, distribution, metabolism and/or excretion of chemicals.

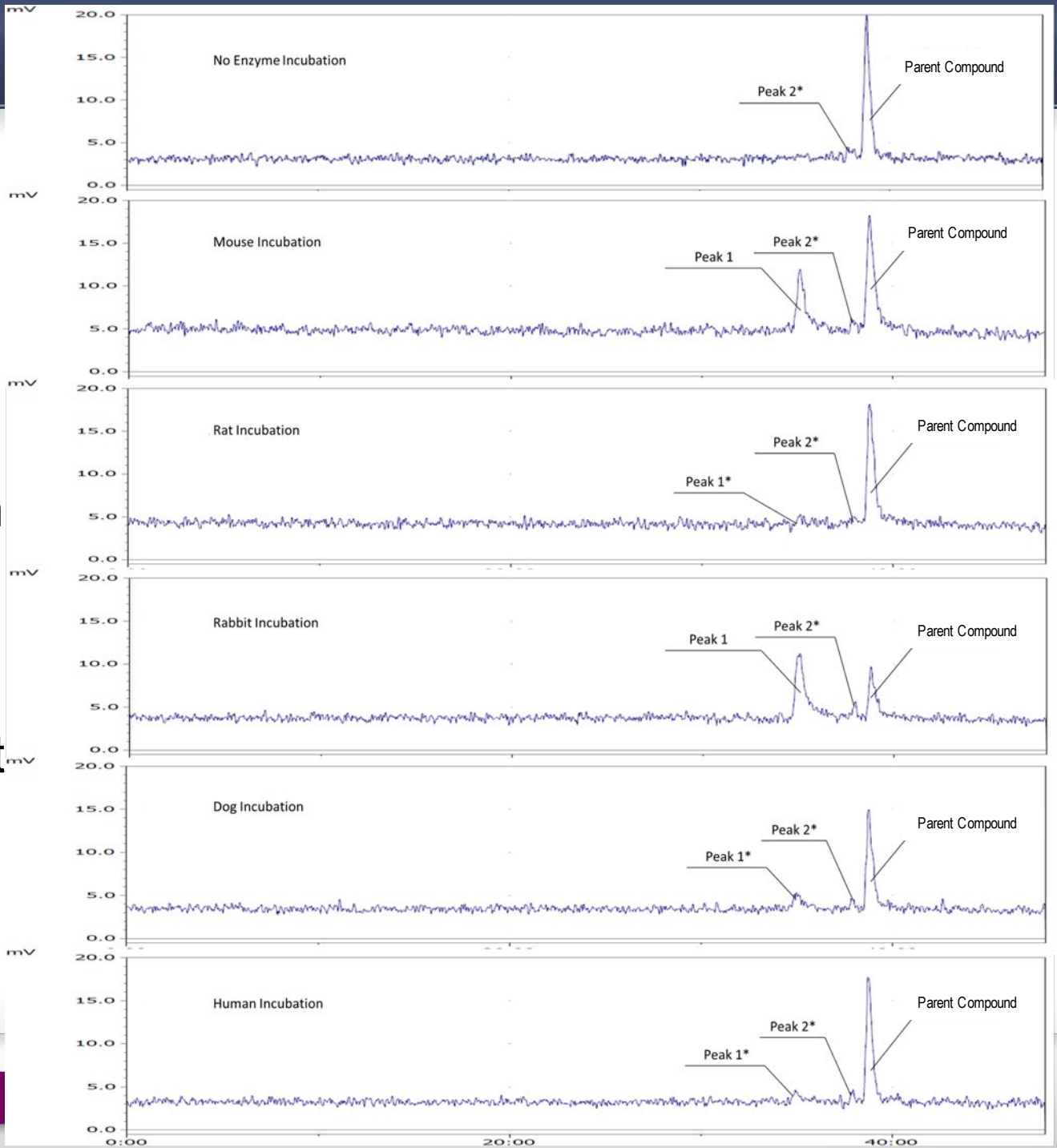


In vitro

Metabolism Studies

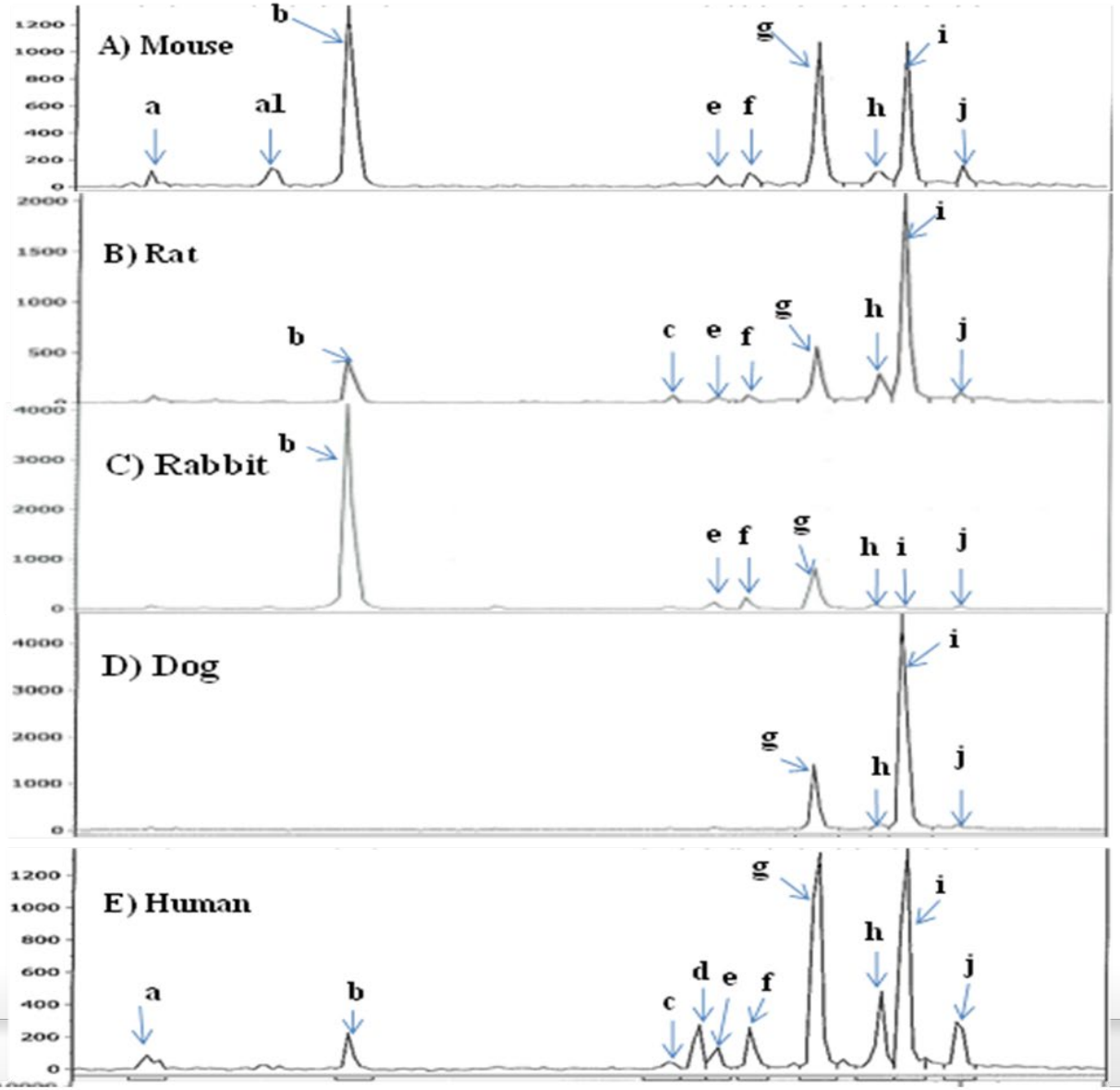
Species Comparisons using liver microsomes

- Identify species with metabolism similar to humans
- In this example, rabbit is not the best model



In vitro Metabolism Studies

- Mice and rats are good models for human metabolism; however, humans have a unique metabolite (peak d) that must be identified and assessed

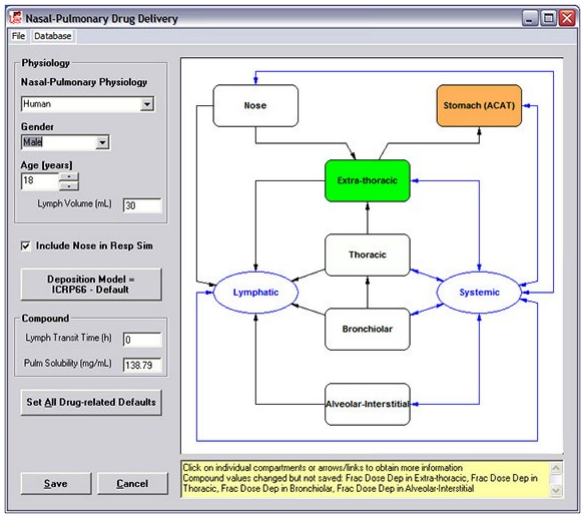


In Silico Toxicokinetics: GastroPlus Modeling

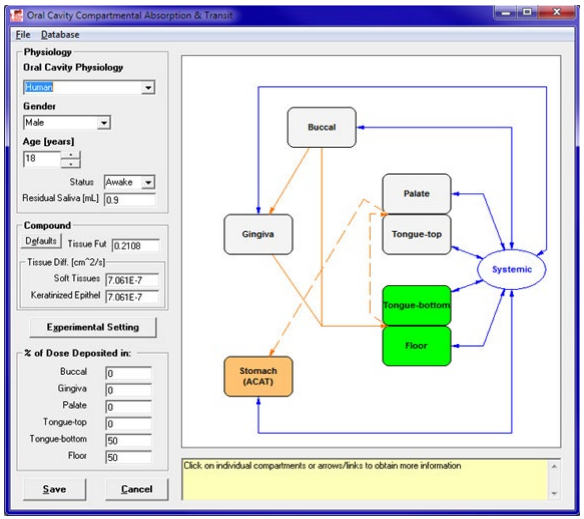
- GastroPlus:
  - Commercially available *in silico* model
  - Predict systemic exposure by multiple routes
    - Oral, inhalation, dermal
  - Predict Absorption, Distribution, Metabolism, Elimination (ADME)
    - Bioavailability
  - Predicts parent compound and metabolite(s)
  - Supports various species and life stages
    - Human and rat

Relevant Exposure Pathways in GastroPlus

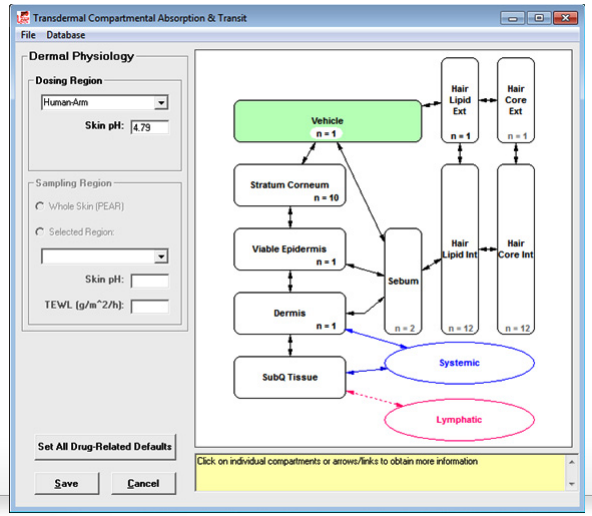
Pulmonary



Oral Cavity

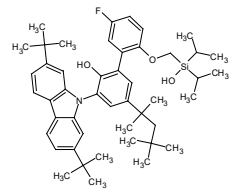
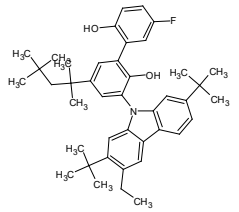
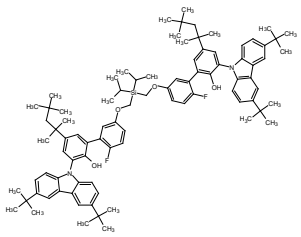
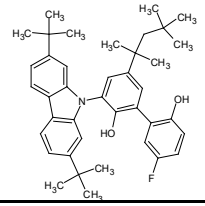


Dermal



- Includes species-specific physiology for multiple species, including humans and rodents.

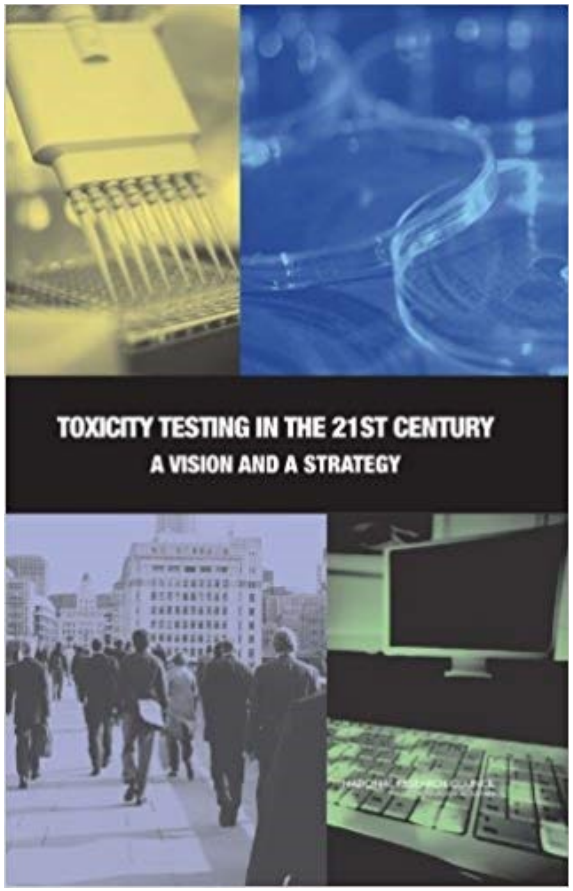
in silico Applications: Toxicity Study Waiver

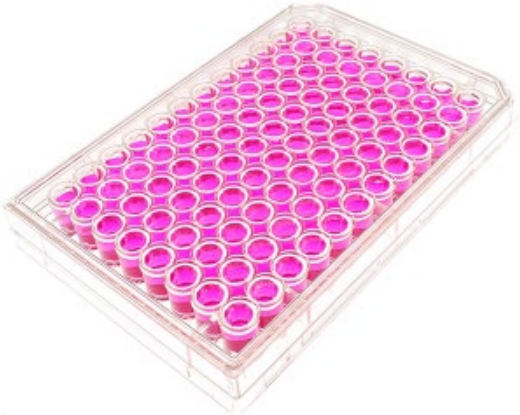
Chemical Name	Structure	Simulation Species	Single Dose Level (mg)	Exposure routes	GastroPlus Simulation Results				
					Molecular Weight	Fa%	F%	Cmax (µg/mL)	AUC $_{0\text{-}168}$ (µg h/mL)
DOC-6194 HL-B		Human (30 years old and body weight 70 kg)	0.0052	Oral	738.12	7.96	1.05	4.66E-08	0.00000420
DOC-6194 HL-E			0.0037		621.88	8.96	0.157	5.33E-08	0.000000559
DOC-6194 Ligand			0.0542		1327.96	0.0542	0.00	0.00	0.00
DOC-6194 HL			0.0173		593.83	11.4	0.972	5.62E-07	0.00000393

Very low bioavailability was predicted and toxicity studies were waived

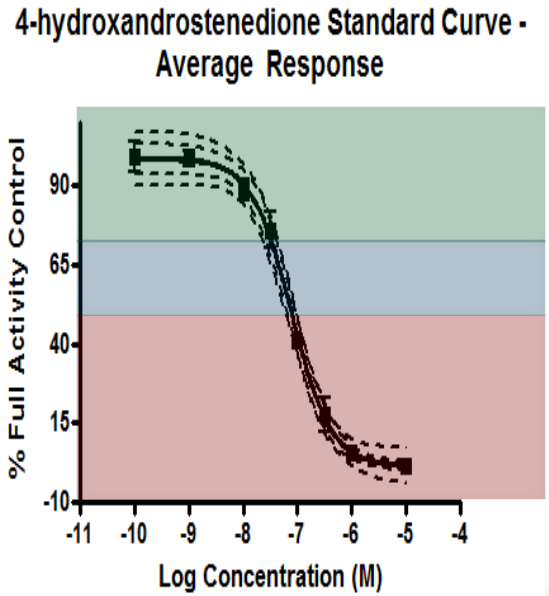
Toxicology in Transition: *In vitro* Screening

In vitro assays used to screen for potential toxicity





Positive – Aromatase Inhibitor

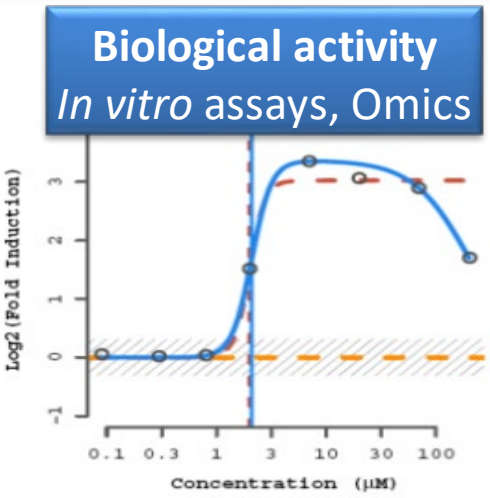


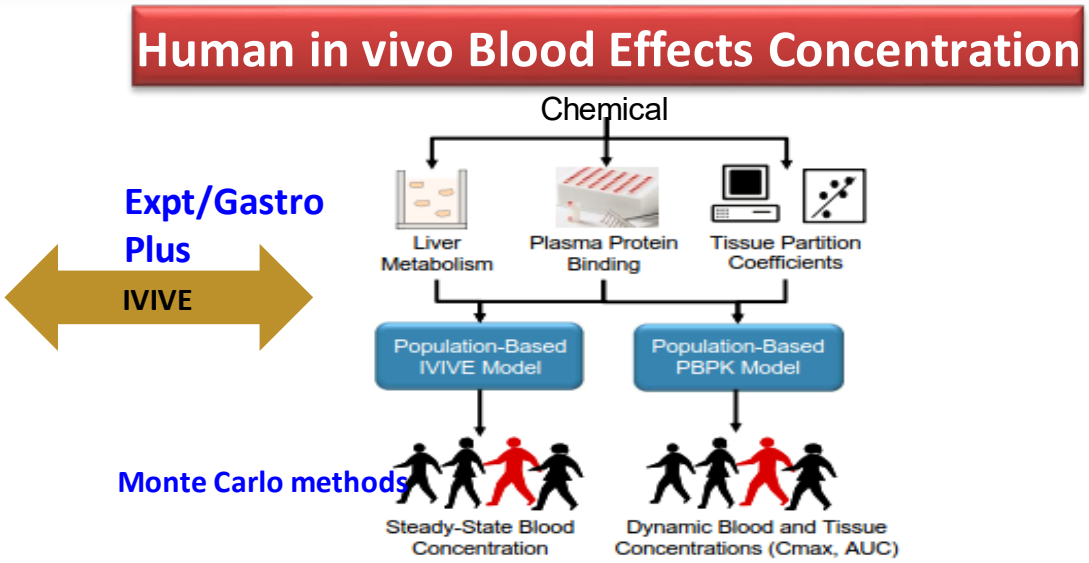
Negative (Non-Inhibitor)

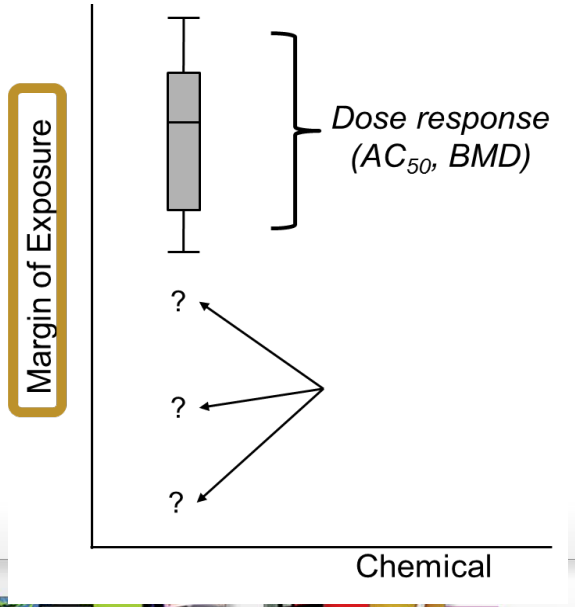
Equivocal

Positive (Inhibitor)

Applications: Human Bioactivity Based MOE



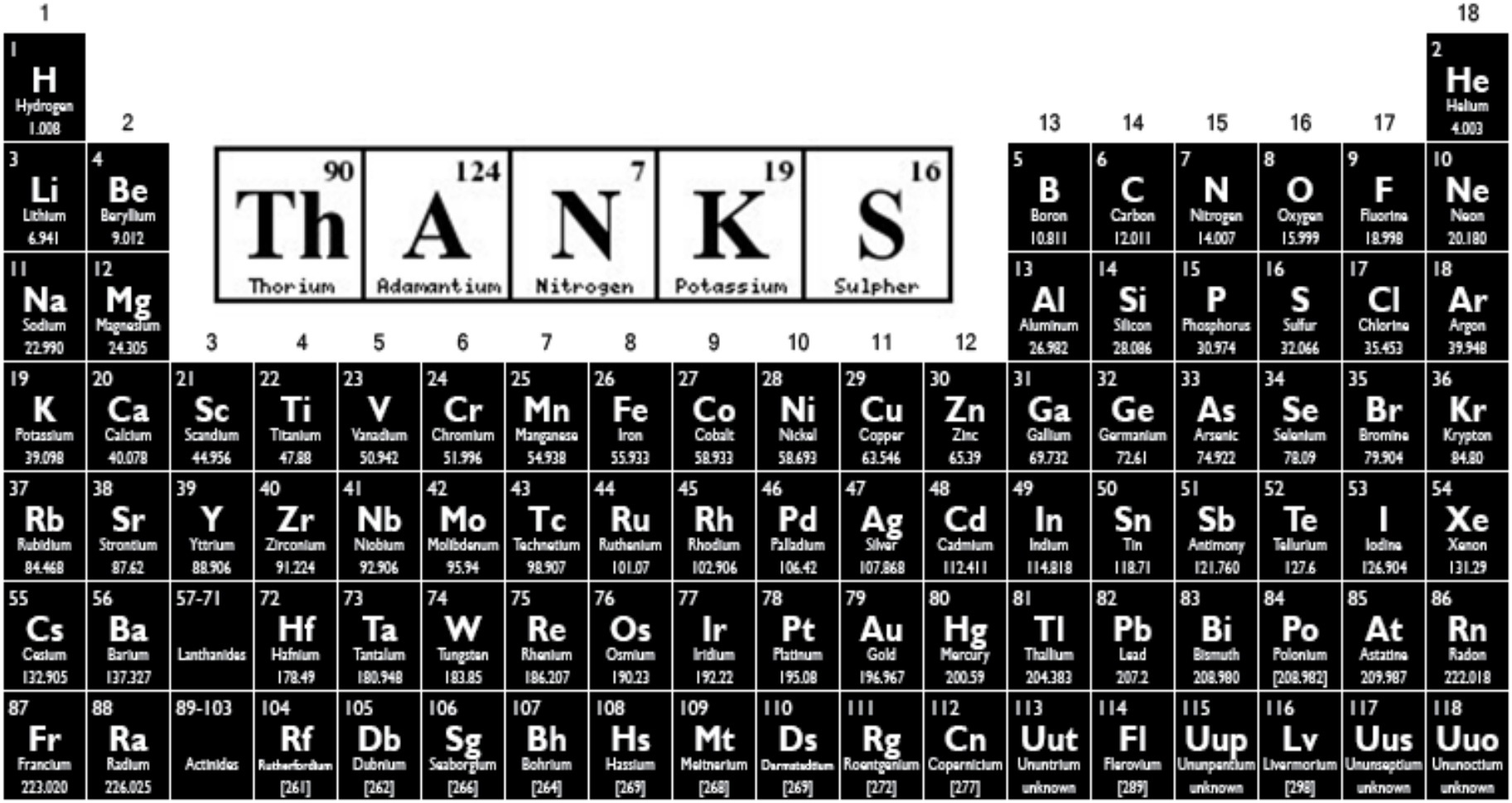




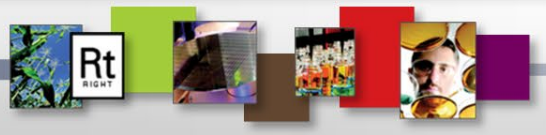
- IVIVE: Estimate exposure to produce *in vitro* "active concentration".
- This "bioactive exposure" is compared with predicted human exposure (margin of exposure)
- Use this MOE to prioritize chemicals for testing

Conclusion

- Toxicokinetics (ADME) play a critical role in understanding toxicity (e.g., dose response)
- TK can be route-, species-, gender-, age- and dose-dependent
- When appropriate (based on exposure), doses in toxicology studies should be in the linear TK range
- *In vitro* TK can be used to establish species relevance
- TK modeling can be used to determine the exposure needed to produce a bioactive internal dose (IVIVE); this exposure can be compared with estimated exposures to determine level of concern


Th 90 Thorium
A 124 Adamantium
N 7 Nitrogen
K 19 Potassium
S 16 Sulpher

EXTRA SLIDES

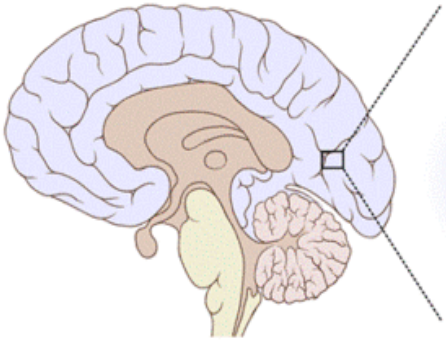


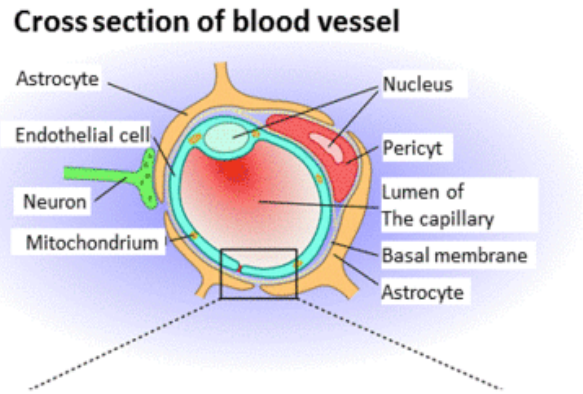
Toxicant Distribution

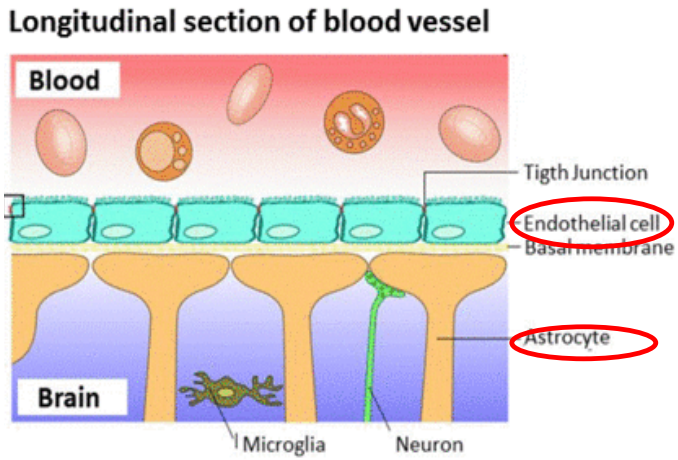
- Rate of distribution to organs/tissues determined by:
  - Blood flow
  - Rate of diffusion from capillaries into tissues (affinity)
- Volume of distribution (Vd): Volume in which the chemical would need to be uniformly dissolved for the observed tissue conc.
    - A chemical that only partitions to plasma has a high plasma concentration and a low Vd
      - e.g., Large molecular weight compounds
    - If a chemical distributes throughout the body has a low plasma concentration and a high Vd
      - Storage sites: Liver/kidney, Fat, Plasma proteins, Bone
      - Toxicity is due to unbound chemical
    - Large Vd can influence chemical $T_{1/2}$
    - Target site storage: ↑ toxicity; Non-target site: protective

Toxicant Distribution

- Blood-Brain Barrier limits access of toxicants to the brain
- Brain deposition: Lipid solubility, low degree of ionization and unbound
- BBB not fully developed at birth (bilirubin encephalopathy)







Excretion

- Many chemicals metabolized to more water-soluble metabolites
- Urinary elimination via kidneys
  - Glomerular filtration (MW ≤ 60 Da; excreted in urine or reabsorbed into blood)
  - Tubular excretion by passive diffusion (minor pathway)
  - Active tubular secretion (e.g., MDR-2,4; OAT-1,2,3)
  - Kidney function is incomplete at birth; therefore, some xenobiotics are eliminated more slowly (e.g., penicillin clearance ~20% in premature infants)
- Fecal elimination
  - Directly transfer from blood to intestinal contents (passive diffusion)
  - Unabsorbed chemicals excreted in feces
  - Biliary elimination (or enterohepatic recirculation due to gut microflora)
- Exhalation
  - Lungs eliminate gas-phase substances and volatile liquids (vapor pressure) via simple diffusion
  - Breathalyzer determination of blood alcohol content