

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

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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 Silico* Approaches to Evaluate TK
- Conclusion



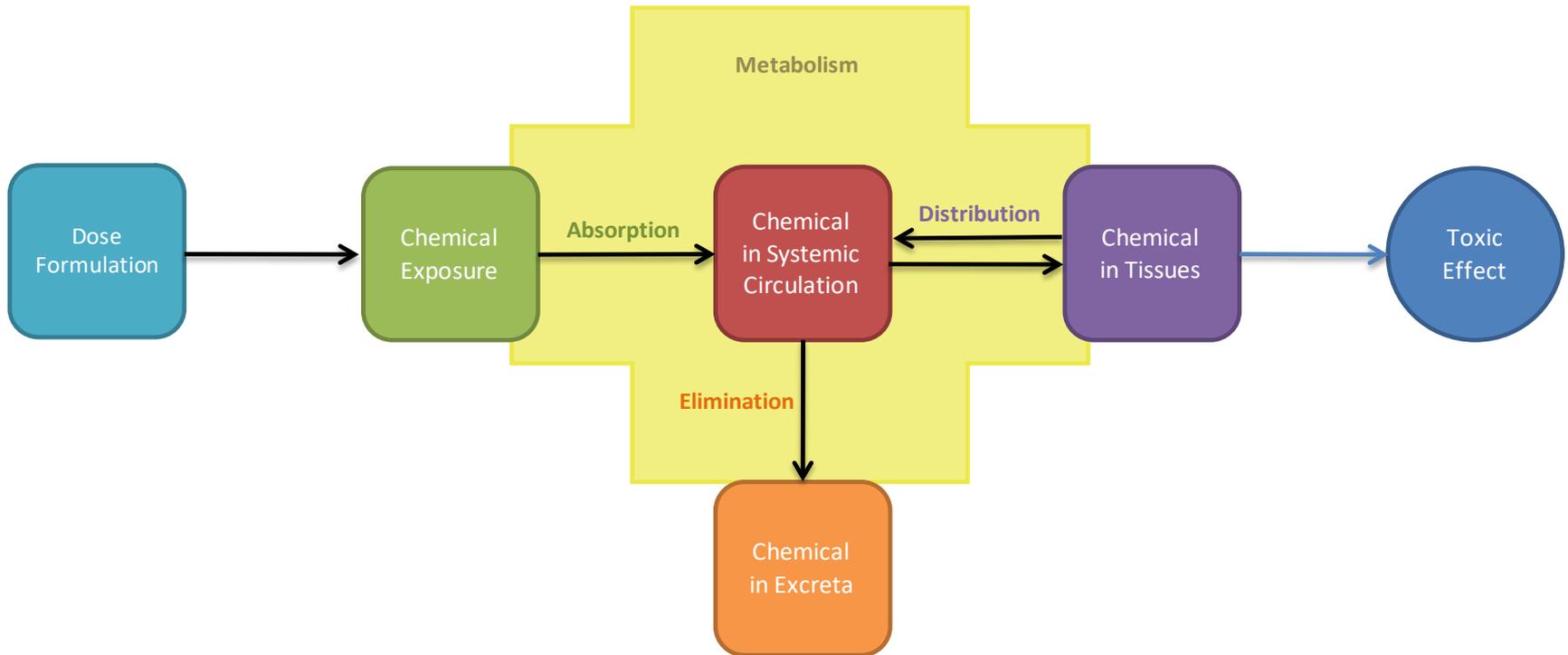
Toxicokinetics

- 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

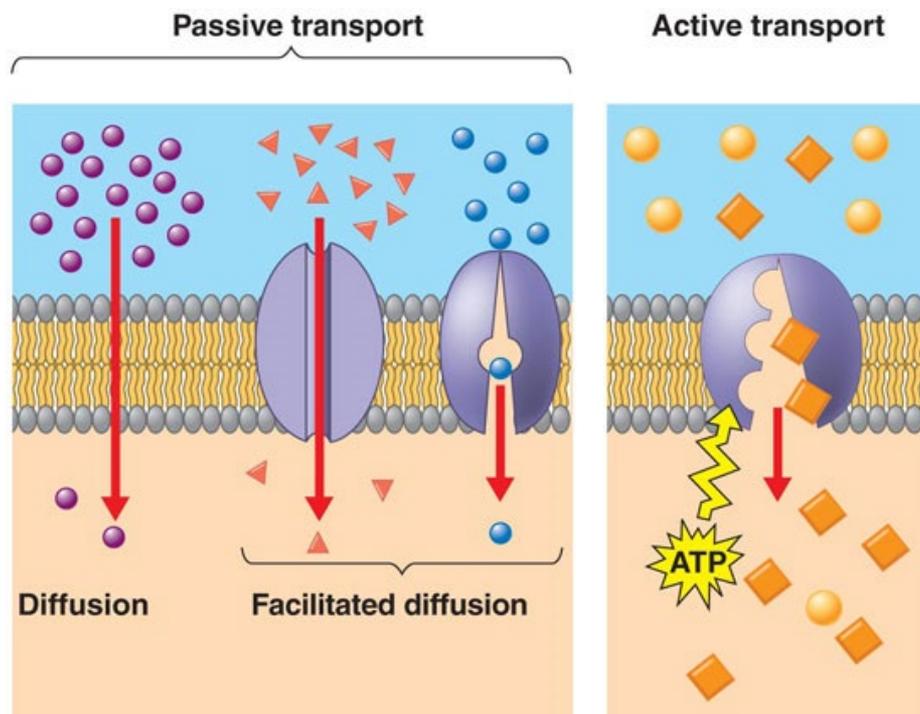


Chemical Factors Favoring Toxicant Absorption

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

} → greater absorption

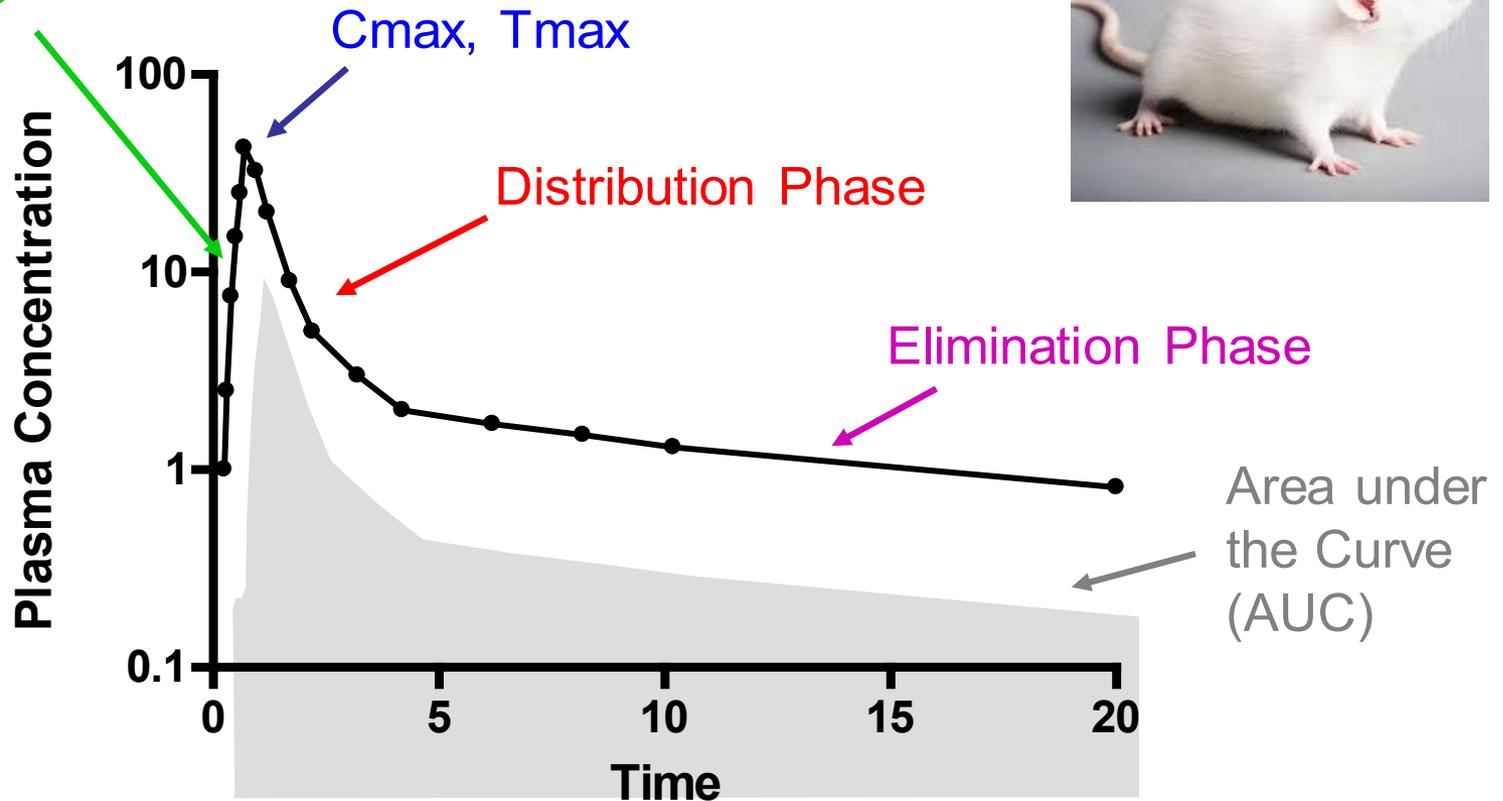
- Movement across membranes by diffusion, facilitated diffusion and active transport



Understanding Toxicokinetics

Typical pharmacokinetic time-course following oral administration

Absorption Phase



Understanding Toxicokinetics

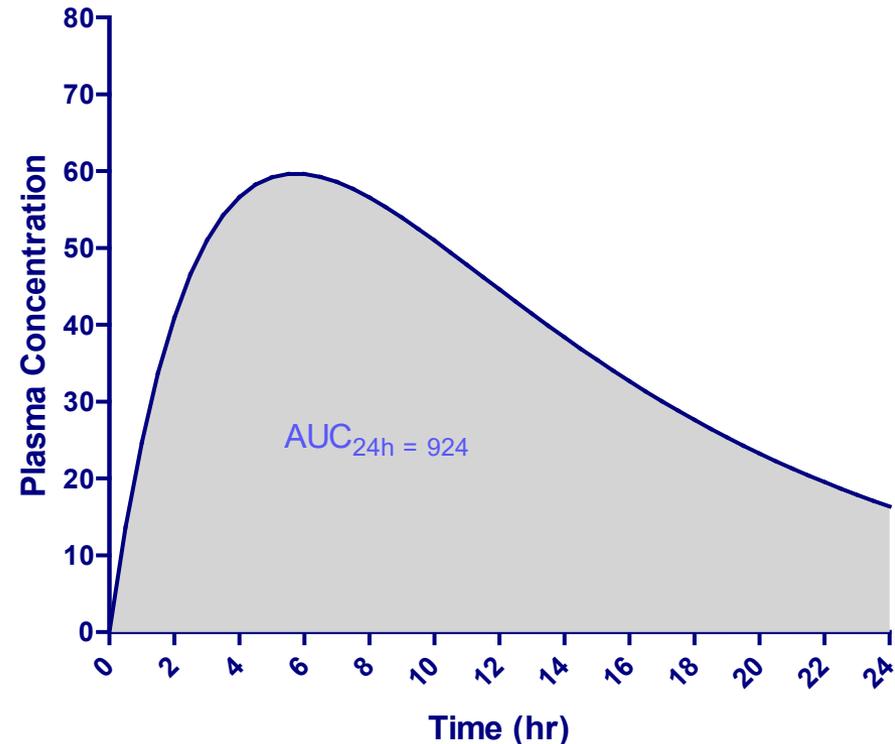
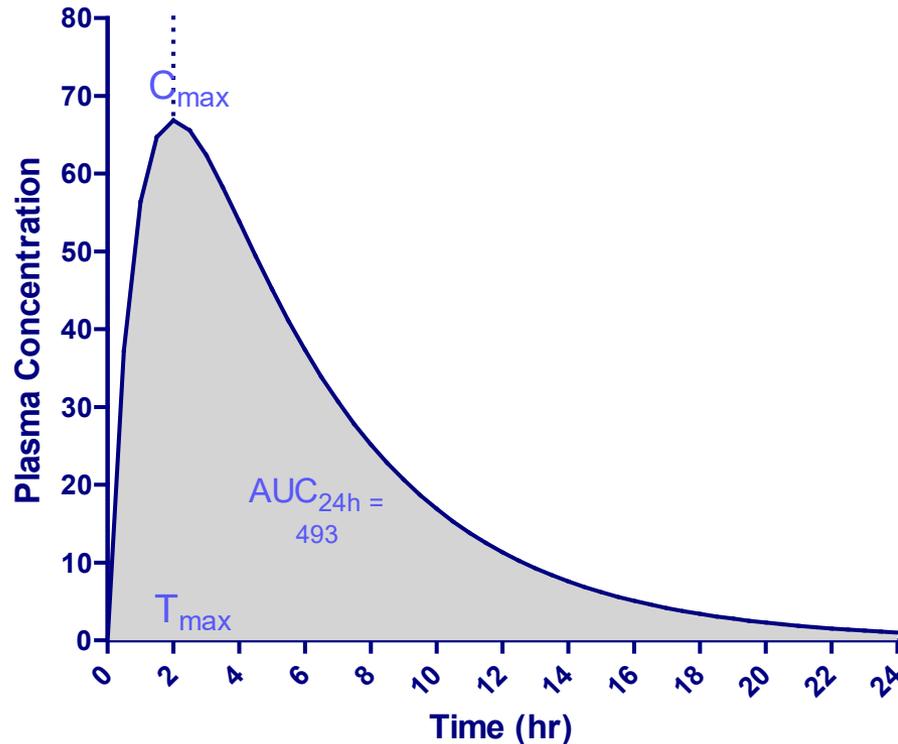
- C_{\max} :
 - Maximum blood/plasma concentration
- T_{\max} :
 - Time of C_{\max}
- AUC_{24h} :
 - Area under the plasma concentration-time curve from 0 – 24 hours
- Half Life ($t_{1/2}$):
 - Time at which half of the dose has been eliminated from the body



Understanding Toxicokinetics

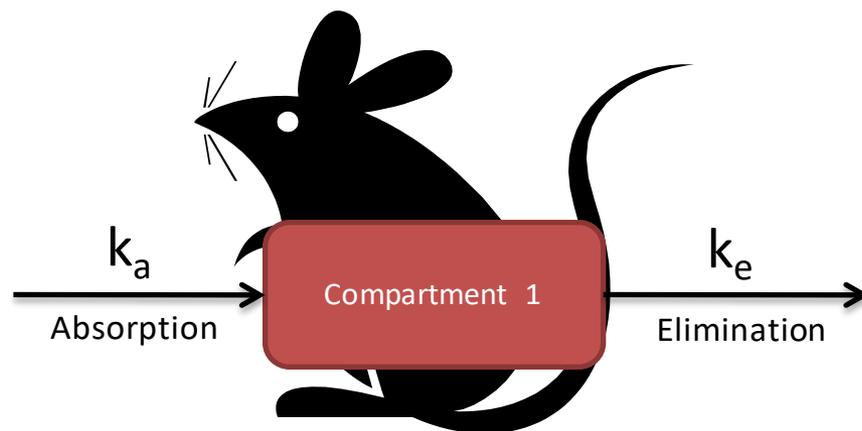
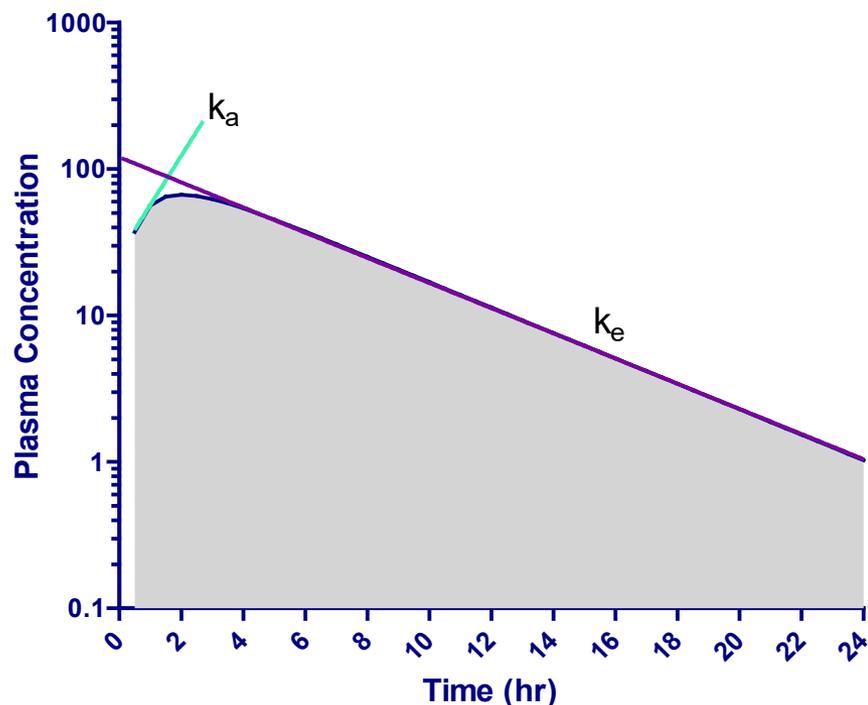
The study of internal exposure over time.

C_{max} : Maximum Concentration and AUC: Area Under the Curve

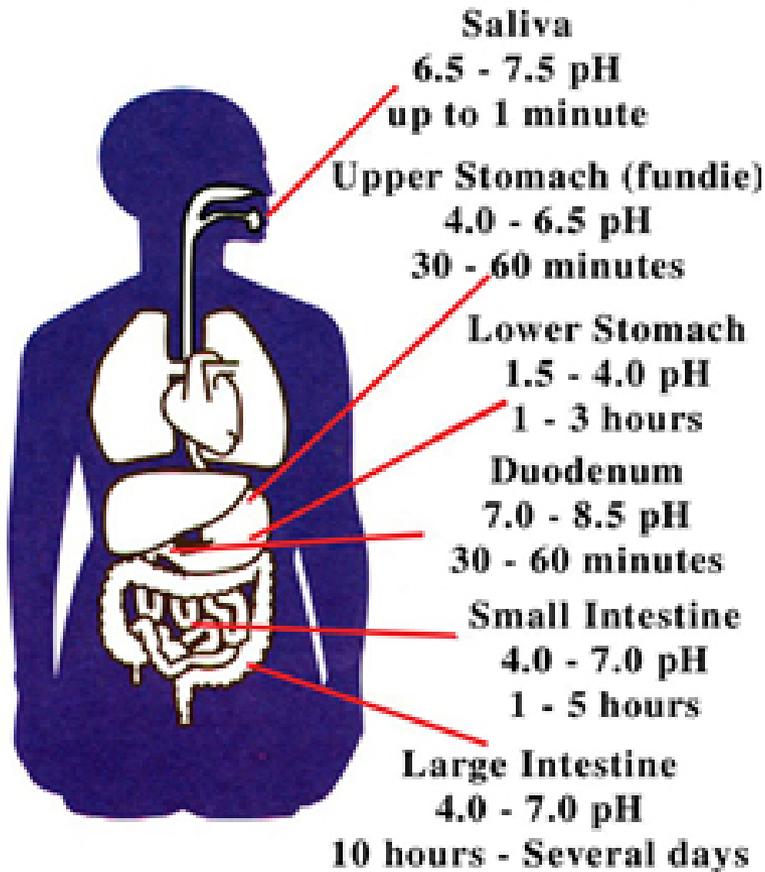


Understanding Toxicokinetics

Toxicity is dependent on absorption, distribution, metabolism and elimination



Toxicokinetics: Route Dependent - Oral

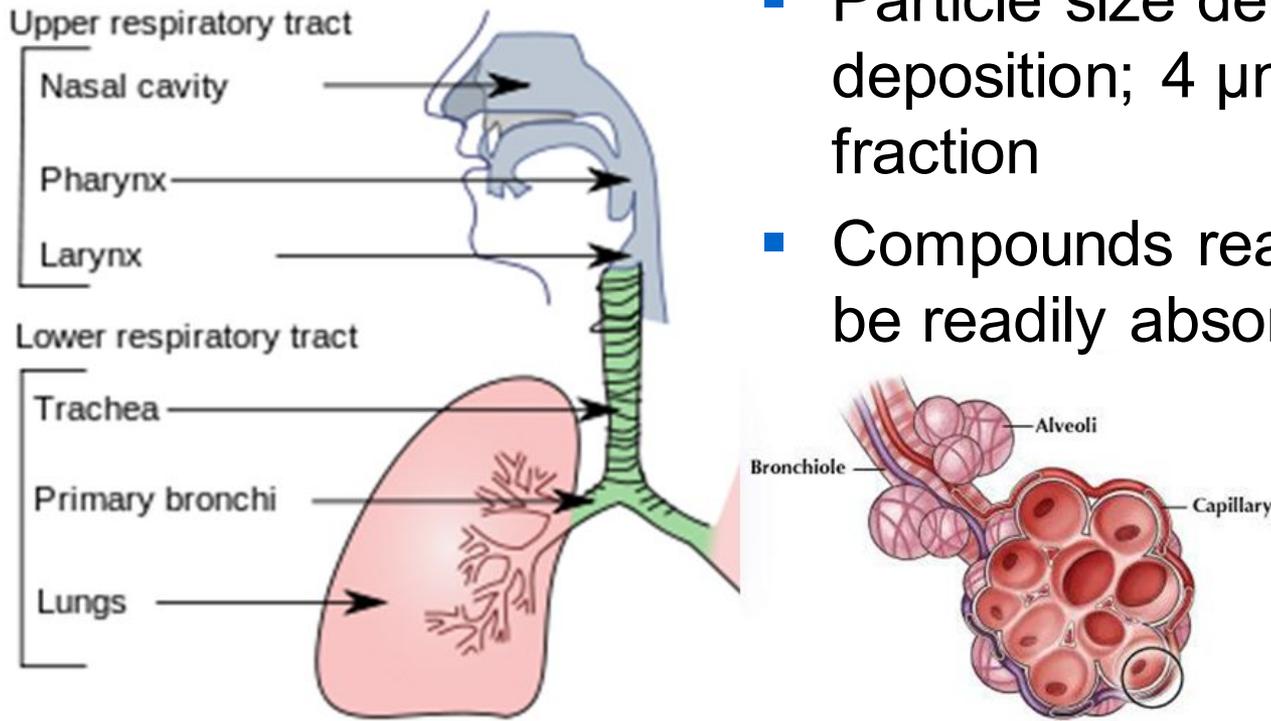


- Compounds can be absorbed throughout the GI tract depending on time spent in contact, surface area and pH.
- Weak acids more easily absorbed in stomach; weak bases in the gi tract (neutral charge)
- Blood from gi to portal vein to liver for biotransformation
 - Detoxification
 - Bioactivation
 - Elimination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

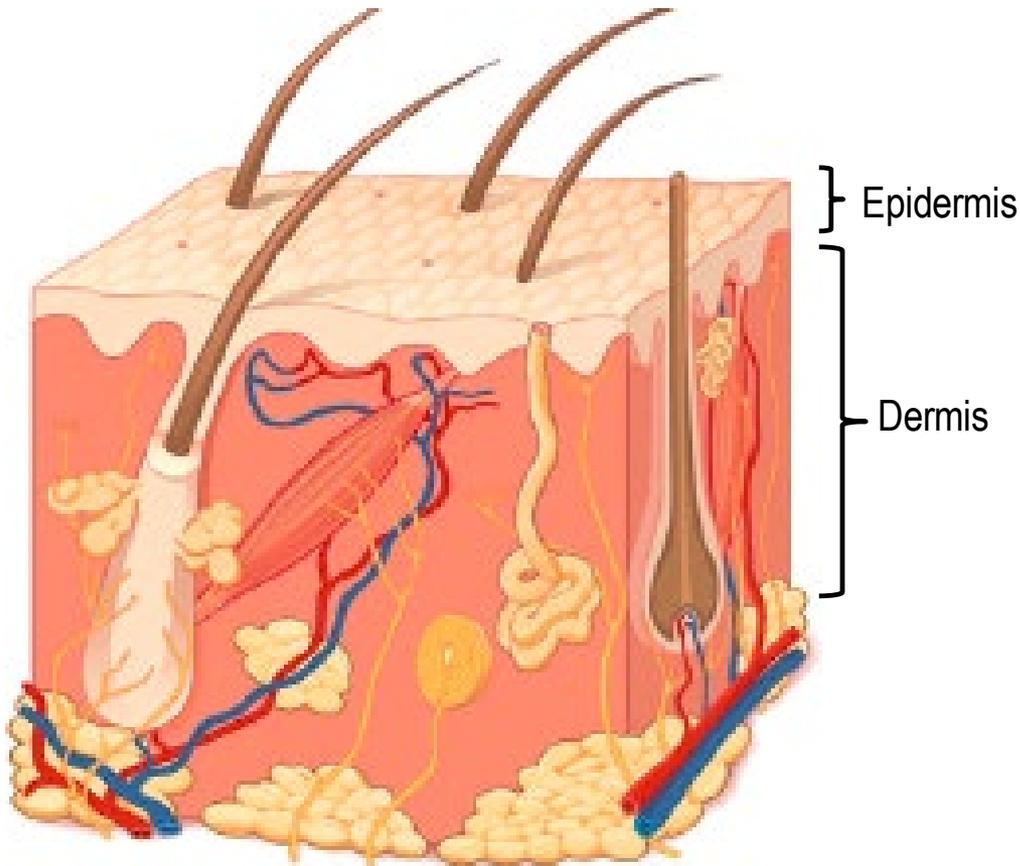


Toxicokinetics: Route Dependent - Respiratory

- 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

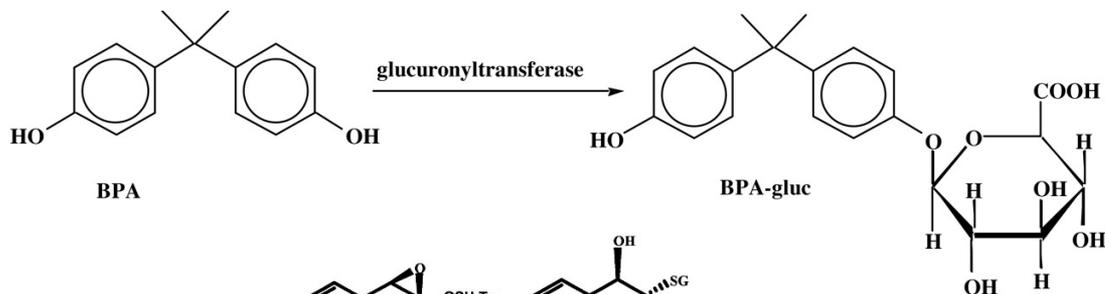
Toxicokinetics: Route of Exposure

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

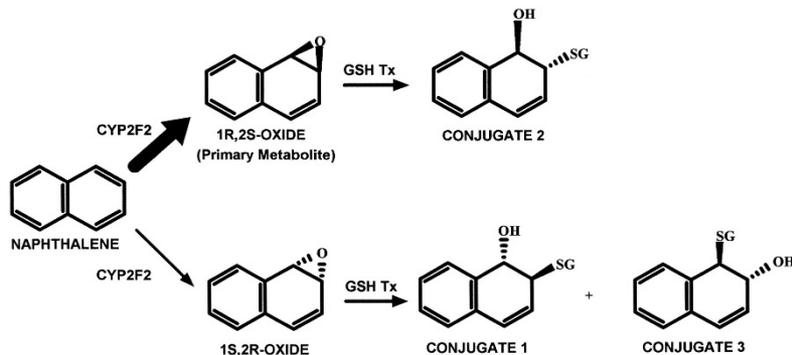


Metabolism: Detoxification or Bioactivation?

- 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.

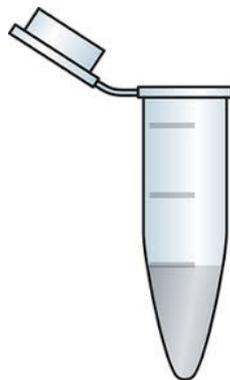


Toxicokinetics Subdisciplines

- 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

4 rats/dose; 1 sex;
≥ 2 dose levels;
¹⁴C-labeled TM;
relevant route and
single/multiple doses

Blood samples at multiple timepts



Excreta samples at multiple timepts



Exhaled air samples for ≥ 24 hr



Measure residual radiolabel in animal

7-day monitoring period

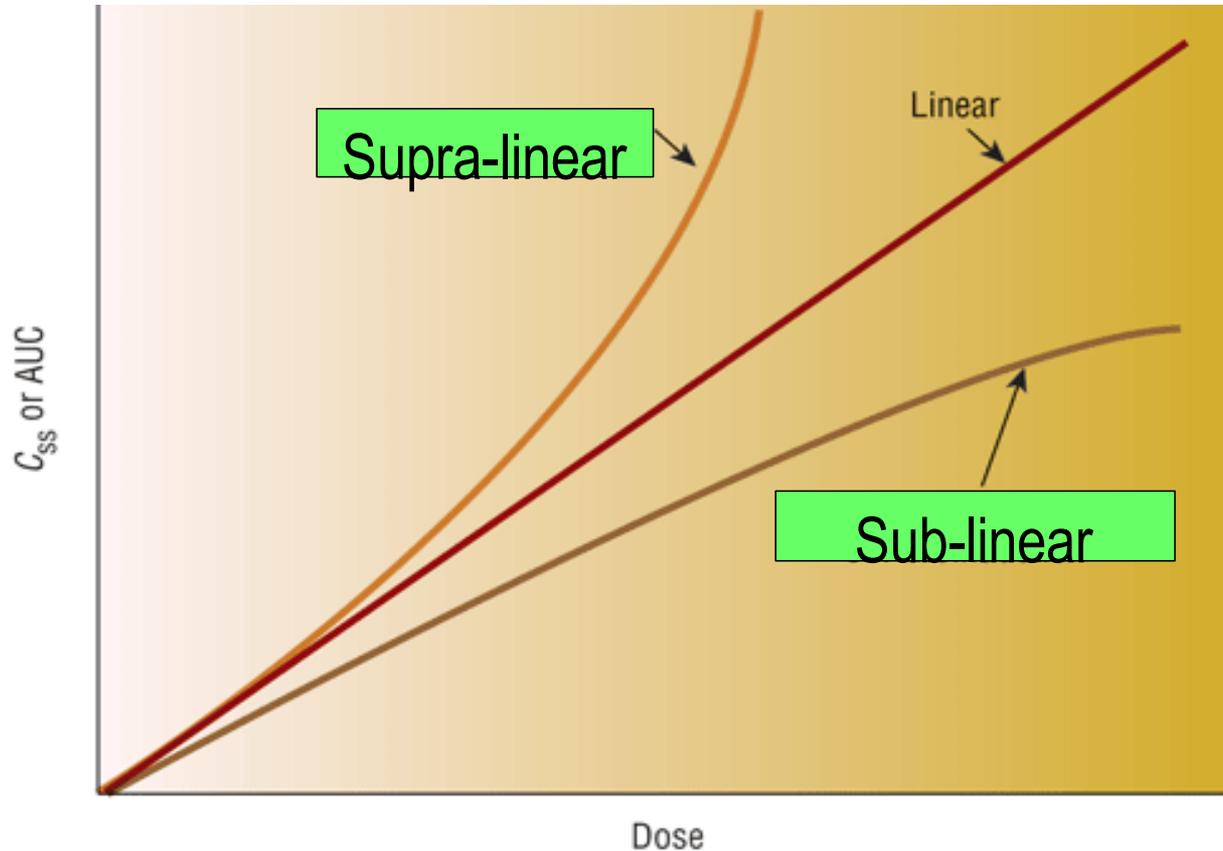
¹⁴C-TM located in metabolically stable portion of the molecule:

- Identification of metabolites with adequate sensitivity
- 90% Mass balance required
- Collect blood at T_{max} for metabolite identification



Toxicokinetic Linearity

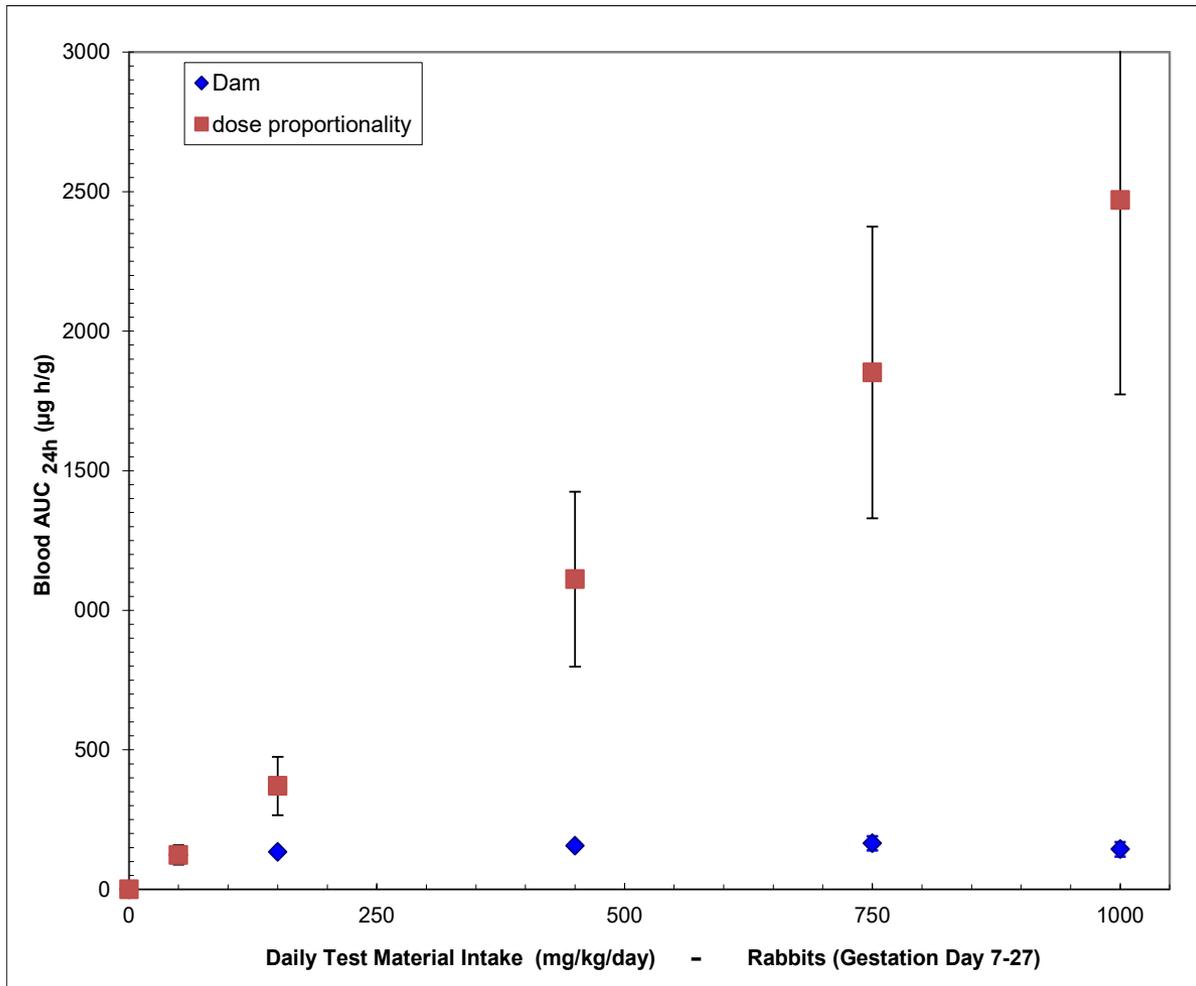
Linear TK is when the internal dose increases proportionally with the applied dose



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



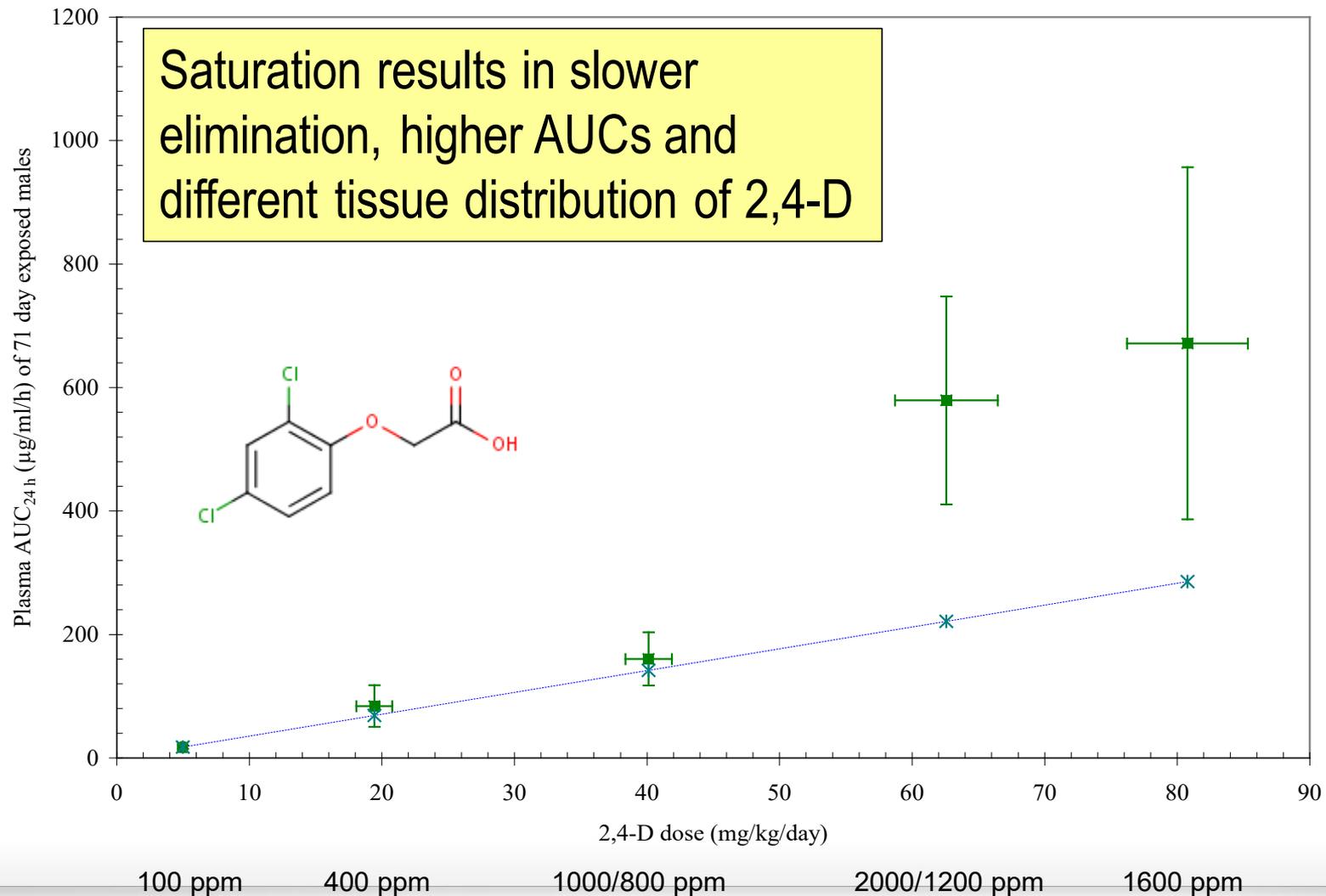
Case Study with Silicones: Saturated Absorption



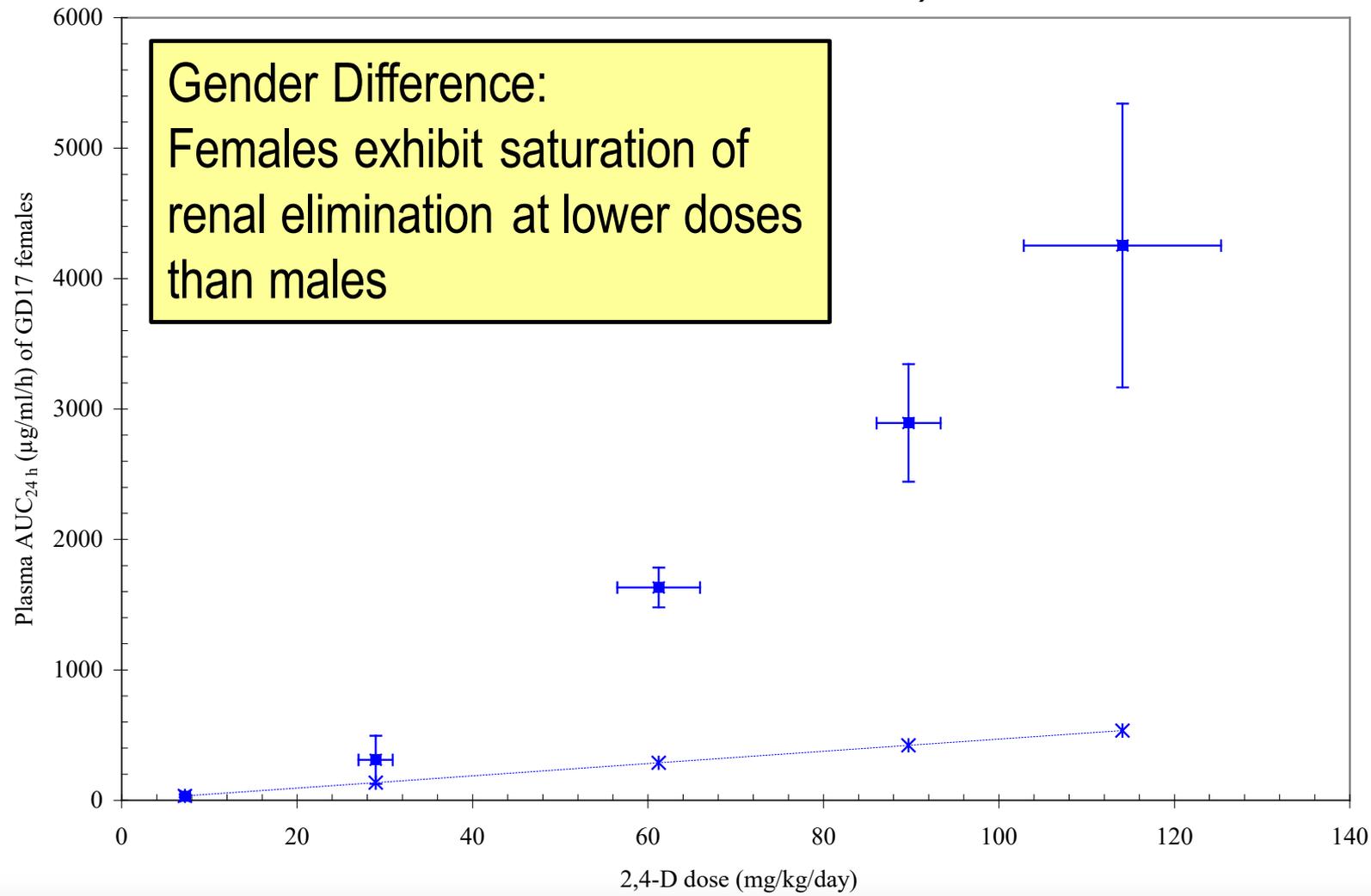
- Higher exposures \neq higher internal dose
- Toxicity may not exhibit dose-response relationships



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)



100 ppm

400 ppm

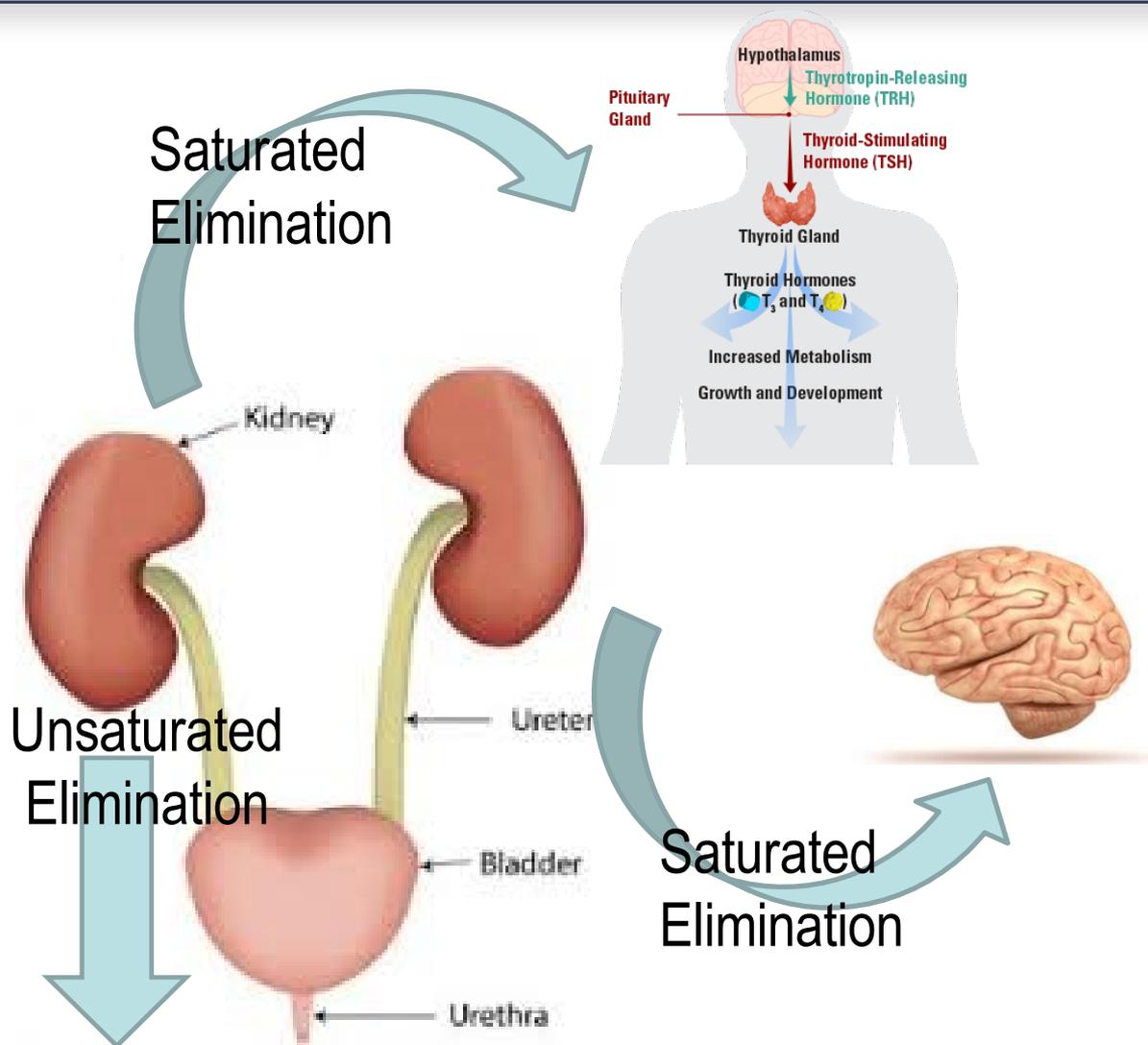
1000/800 ppm

2000/1200 ppm

1600 ppm

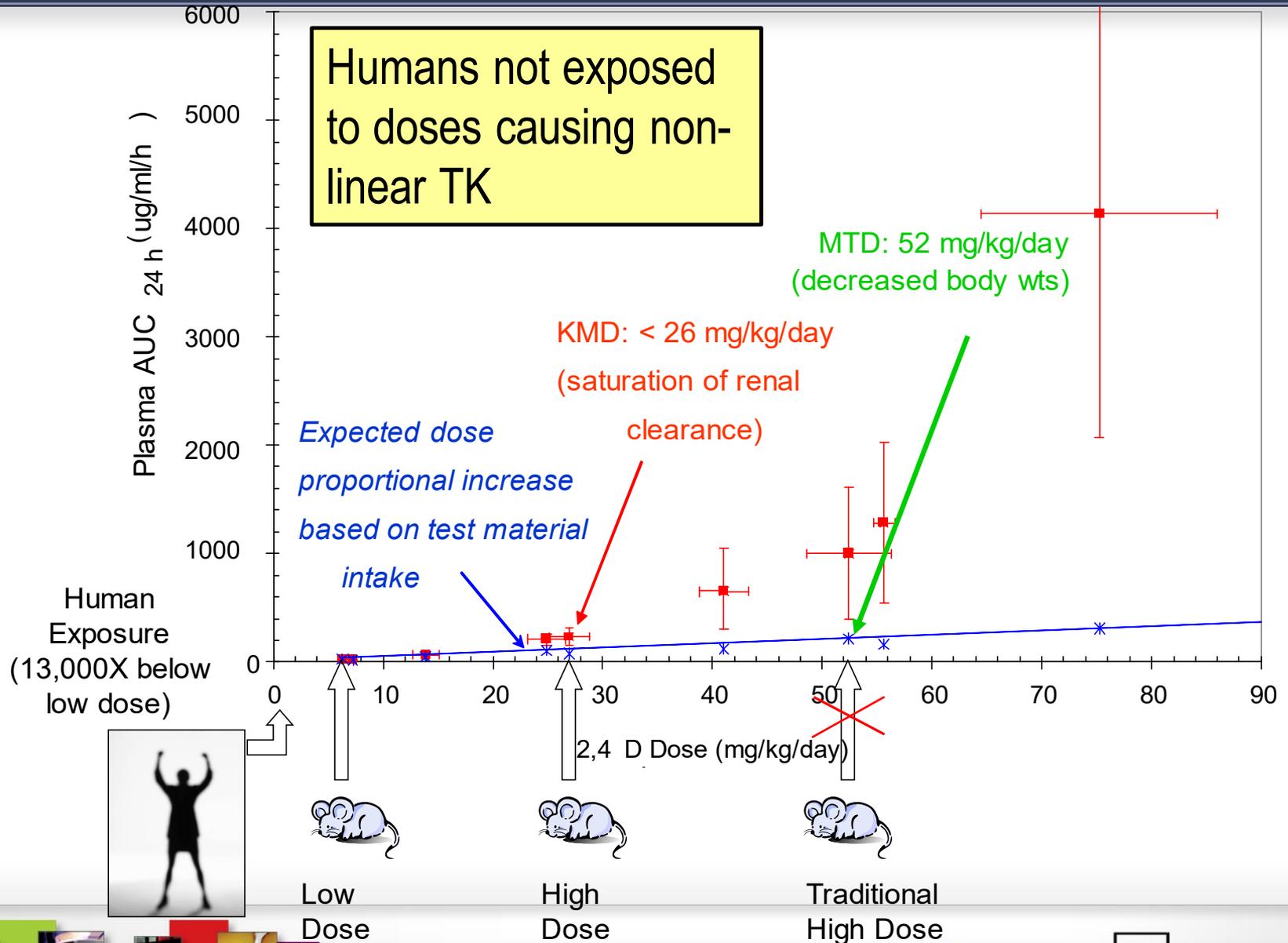


2,4-D Tissue Distribution with Nonlinear TK



- 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

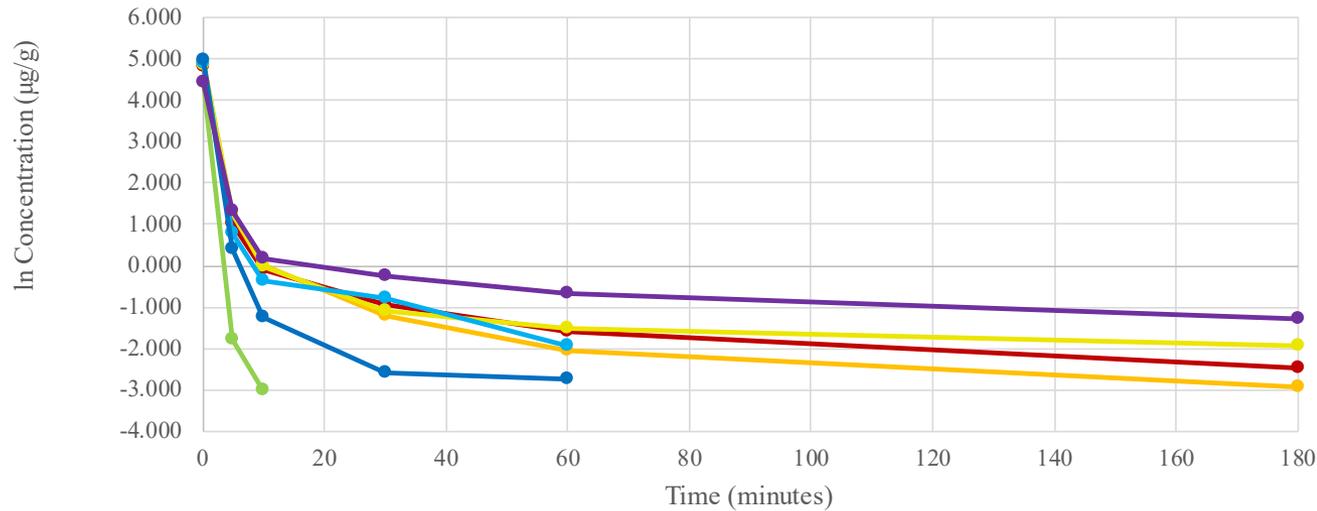


Toxicokinetics Parameters

- 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

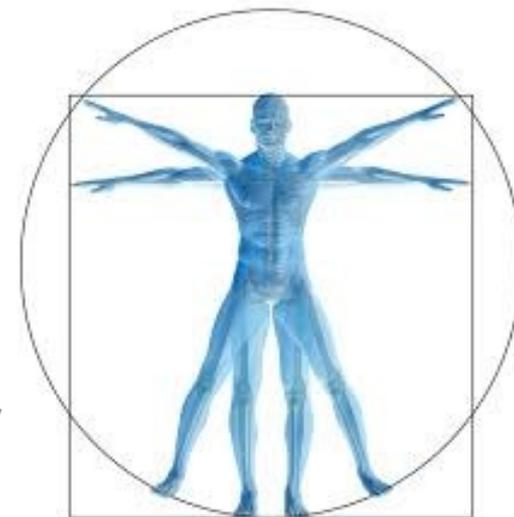
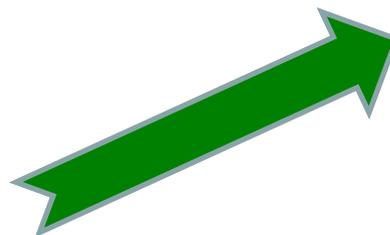
‘Read Across’ for Related Chemistries:

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



Predictive Toxicokinetics

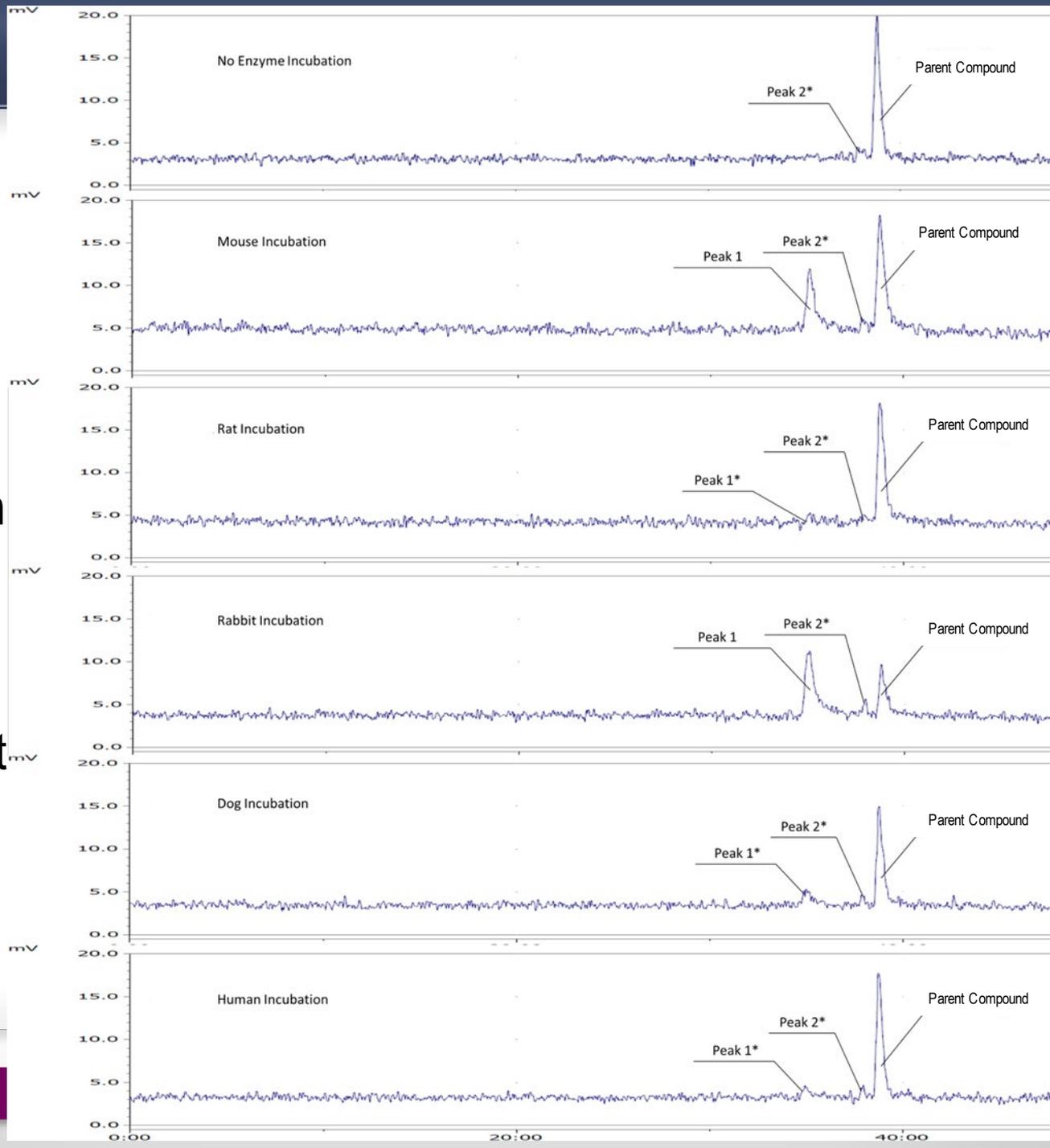
Using *in vitro* or *in silico* models to predict absorption, distribution, metabolism and/or excretion of chemicals.



In vitro Metabolism Studies

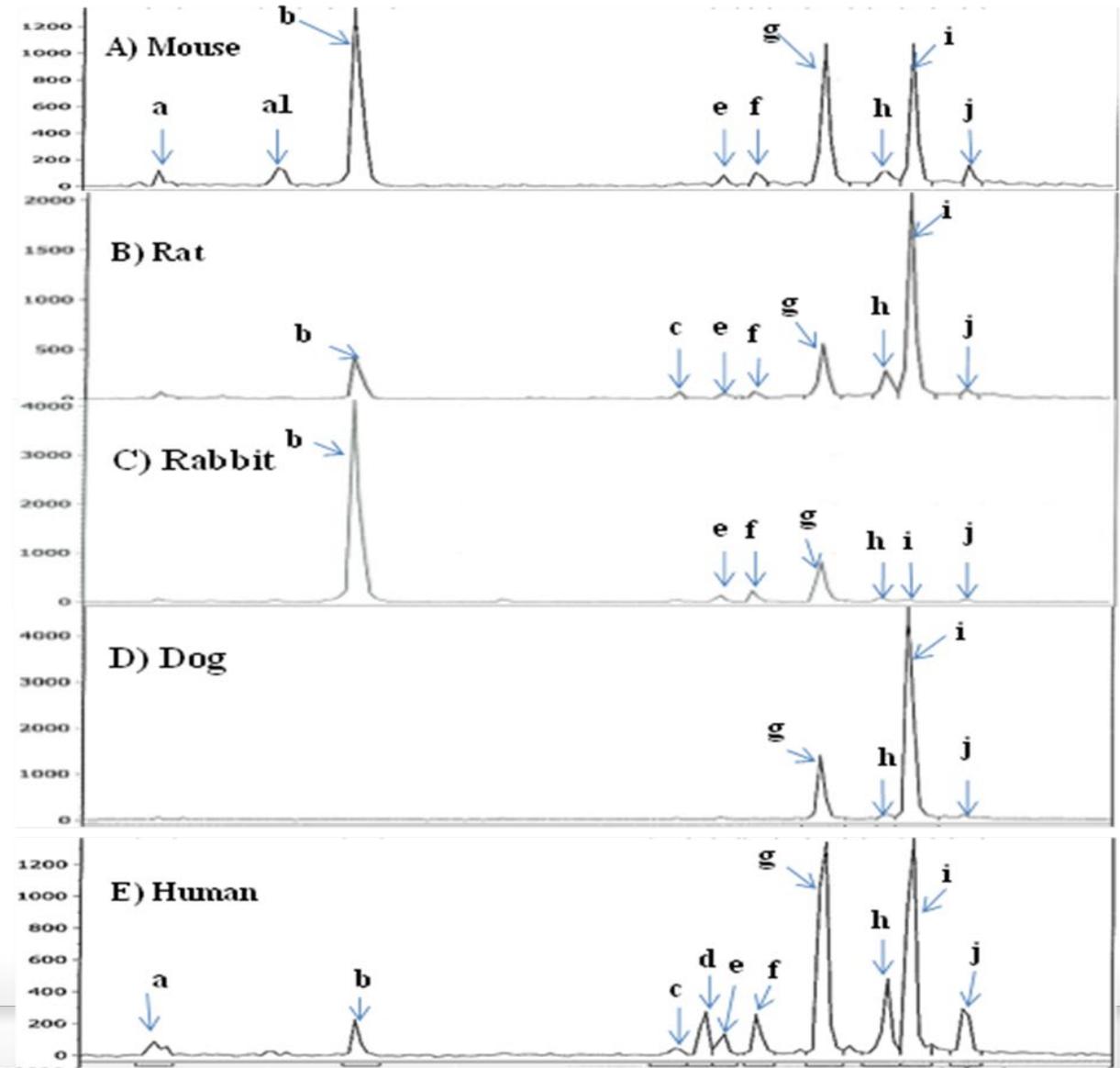
Species Comparisons
using liver microsomes

- 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



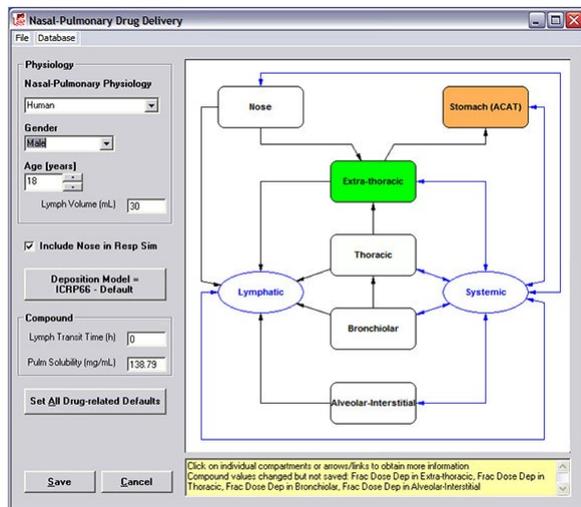
In Silico Toxicokinetics: GastroPlus Modeling

- 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

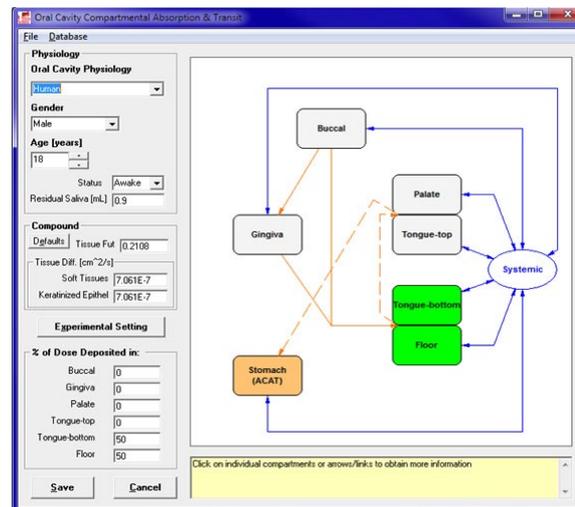


Relevant Exposure Pathways in GastroPlus

Pulmonary

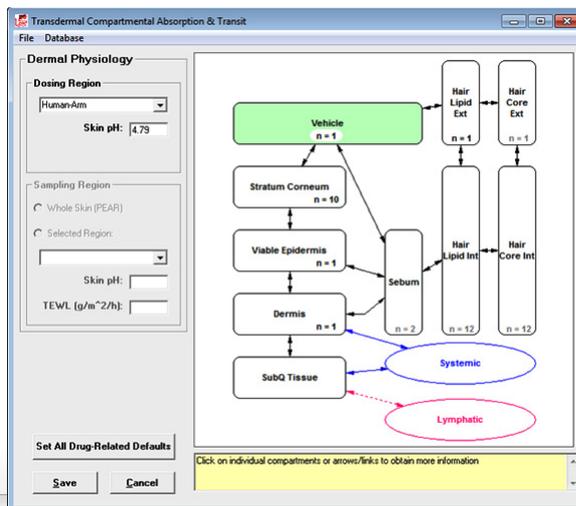


Oral Cavity

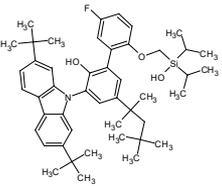
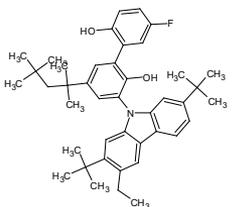
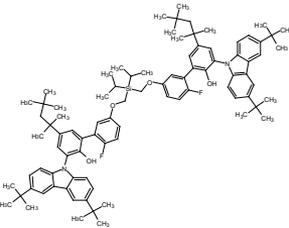
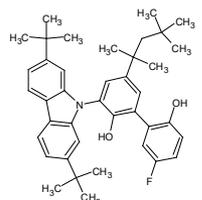


Dermal

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



in silico Applications: Toxicity Study Waiver

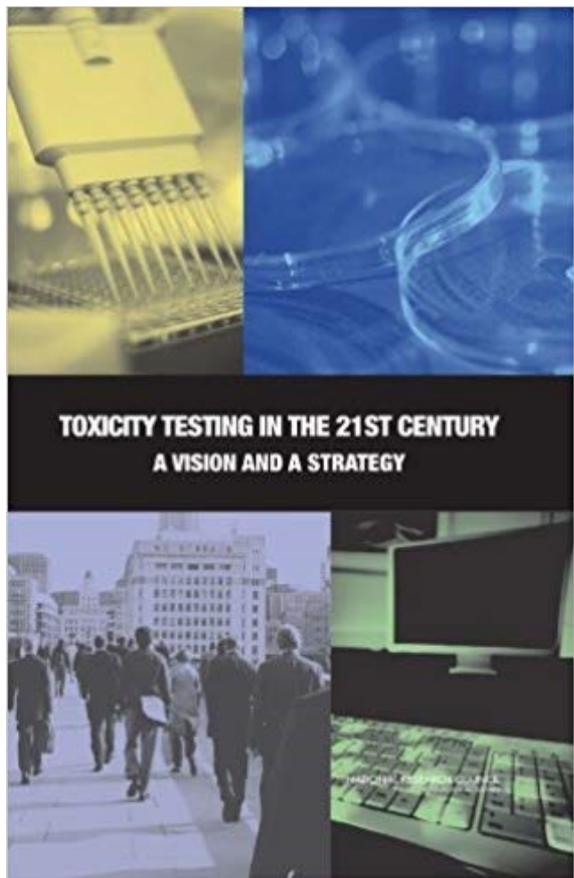
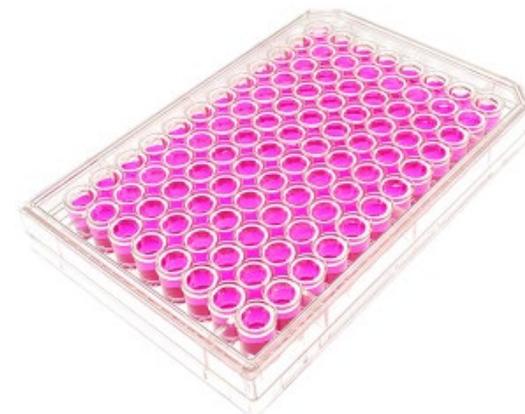
Chemical Name	Structure	Simulation Species	Single Dose Level (mg)	Exposure routes	GastroPlus Simulation Results				
					Molecular Weight	Fa %	F %	Cmax (µg/mL)	AUC ₀₋₁₆₈ (µg h/mL)
DOC-6194 HL-B		Human (30 years old and body weight 70 kg)	0.0052	Oral	738.12	7.96	1.05	4.66E-08	0.00000420
DOC-6194 HL-E			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



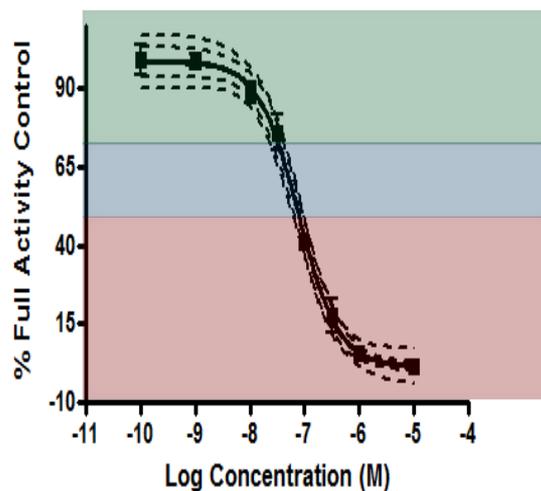
Toxicology in Transition: *In vitro* Screening

In vitro assays used to screen for potential toxicity



Positive – Aromatase Inhibitor

4-hydroxandrostenedione Standard Curve - Average Response



Negative (Non-Inhibitor)

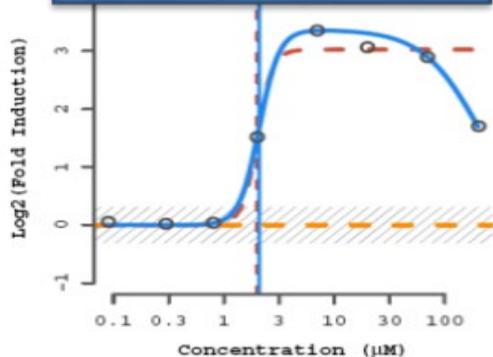
Equivocal

Positive (Inhibitor)



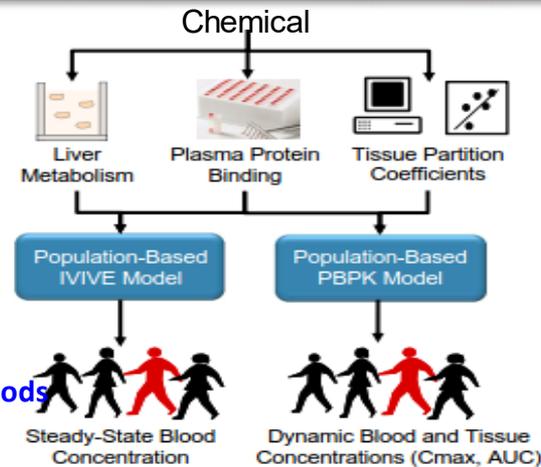
Applications: Human Bioactivity Based MOE

Biological activity *In vitro* assays, Omics

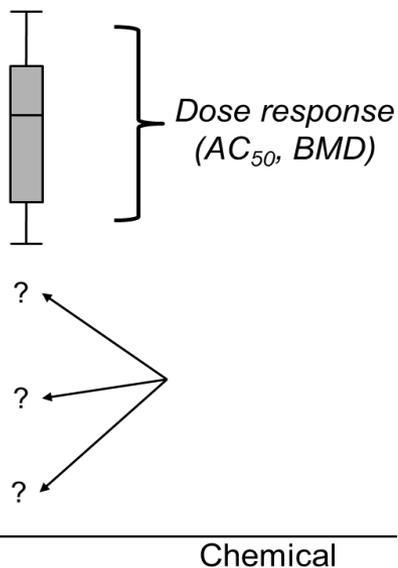


Human in vivo Blood Effects Concentration

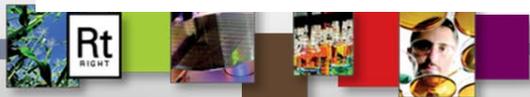
Expt/Gastro
Plus
IVIVE



Margin of Exposure



- 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



1													18													
1 H Hydrogen 1.008																		2 He Helium 4.003								
3 Li Lithium 6.941	4 Be Beryllium 9.012	<table border="1" style="margin: auto;"> <tr> <td>90 Th Thorium</td> <td>124 A Adamantium</td> <td>7 N Nitrogen</td> <td>19 K Potassium</td> <td>16 S Sulphur</td> </tr> </table>														90 Th Thorium	124 A Adamantium	7 N Nitrogen	19 K Potassium	16 S Sulphur	5 B Boron 10.811	6 C Carbon 12.011	7 N Nitrogen 14.007	8 O Oxygen 15.999	9 F Fluorine 18.998	10 Ne Neon 20.180
90 Th Thorium	124 A Adamantium	7 N Nitrogen	19 K Potassium	16 S Sulphur																						
11 Na Sodium 22.990	12 Mg Magnesium 24.305	13 Al Aluminum 26.982	14 Si Silicon 28.086	15 P Phosphorus 30.974	16 S Sulfur 32.066	17 Cl Chlorine 35.453	18 Ar Argon 39.948																			
19 K Potassium 39.098	20 Ca Calcium 40.078	21 Sc Scandium 44.956	22 Ti Titanium 47.88	23 V Vanadium 50.942	24 Cr Chromium 51.996	25 Mn Manganese 54.938	26 Fe Iron 55.933	27 Co Cobalt 58.933	28 Ni Nickel 58.693	29 Cu Copper 63.546	30 Zn Zinc 65.39	31 Ga Gallium 69.732	32 Ge Germanium 72.61	33 As Arsenic 74.922	34 Se Selenium 78.09	35 Br Bromine 79.904	36 Kr Krypton 84.80									
37 Rb Rubidium 84.468	38 Sr Strontium 87.62	39 Y Yttrium 88.906	40 Zr Zirconium 91.224	41 Nb Niobium 92.906	42 Mo Molybdenum 95.94	43 Tc Technetium 98.907	44 Ru Ruthenium 101.07	45 Rh Rhodium 102.906	46 Pd Palladium 106.42	47 Ag Silver 107.868	48 Cd Cadmium 112.411	49 In Indium 114.818	50 Sn Tin 118.71	51 Sb Antimony 121.760	52 Te Tellurium 127.6	53 I Iodine 126.904	54 Xe Xenon 131.29									
55 Cs Cesium 132.905	56 Ba Barium 137.327	57-71 Lanthanides	72 Hf Hafnium 178.49	73 Ta Tantalum 180.948	74 W Tungsten 183.85	75 Re Rhenium 186.207	76 Os Osmium 190.23	77 Ir Iridium 192.22	78 Pt Platinum 195.08	79 Au Gold 196.967	80 Hg Mercury 200.59	81 Tl Thallium 204.383	82 Pb Lead 207.2	83 Bi Bismuth 208.980	84 Po Polonium [208.982]	85 At Astatine 209.987	86 Rn Radon 222.018									
87 Fr Francium 223.020	88 Ra Radium 226.025	89-103 Actinides	104 Rf Rutherfordium [261]	105 Db Dubnium [262]	106 Sg Seaborgium [266]	107 Bh Bohrium [264]	108 Hs Hassium [269]	109 Mt Meitnerium [268]	110 Ds Darmstadtium [269]	111 Rg Roentgenium [272]	112 Cn Copernicium [277]	113 Uut Ununtrium unknown	114 Fl Flerovium [289]	115 Uup Ununpentium unknown	116 Lv Livermorium [293]	117 Uus Ununseptium unknown	118 Uuo Ununoctium unknown									



EXTRA SLIDES



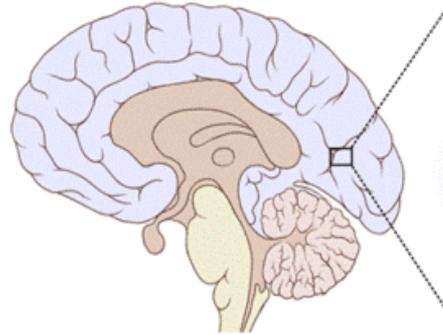
Toxicant Distribution

- 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

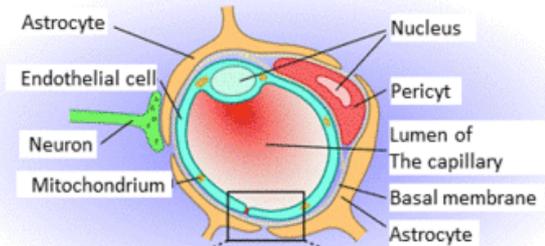


Toxicant Distribution

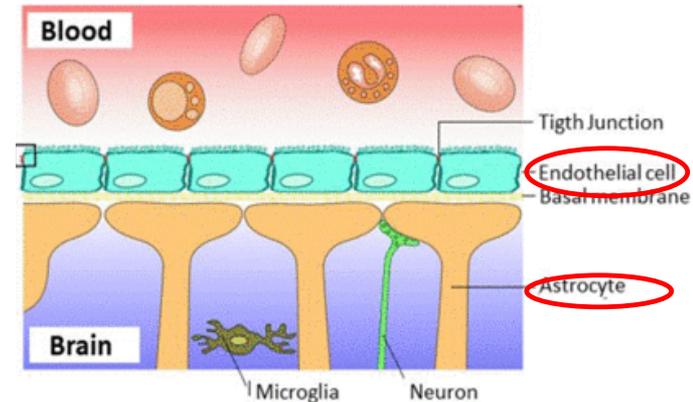
- Blood-Brain Barrier limits access of toxicants to the brain
- Brain deposition: Lipid solubility, low degree of ionization and unbound
- BBB not fully developed at birth (bilirubin encephalopathy)



Cross section of blood vessel



Longitudinal section of blood vessel



Excretion

- Many chemicals metabolized to more water-soluble metabolites
- Urinary elimination via kidneys
 - Glomerular filtration ($MW \leq 60$ 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 $\sim 20\%$ 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

