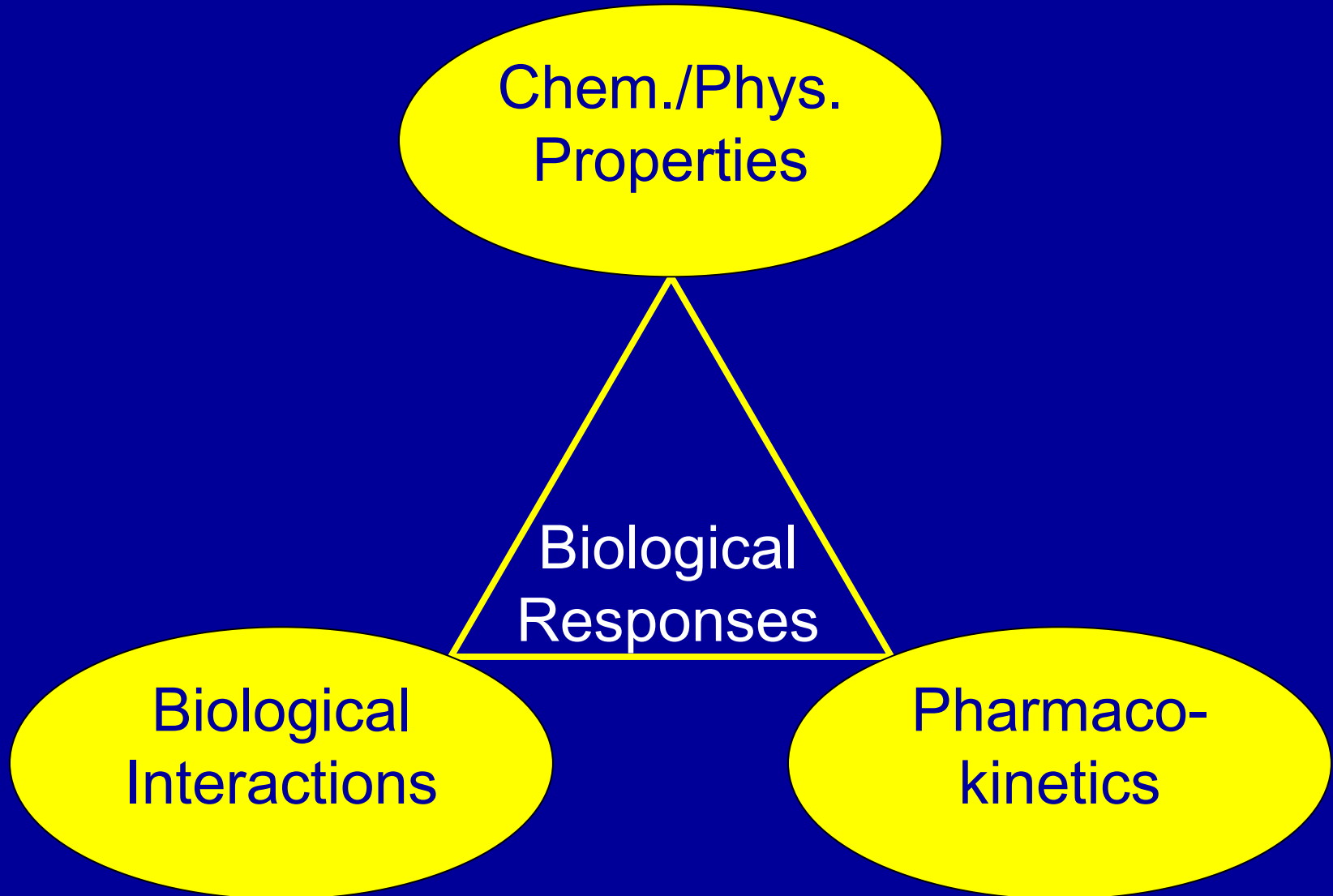


# MECHANISTIC AND PHARMACOKINETIC DETERMINANTS OF PERFLUOROALKYL TOXICITY

John L. Butenhoff, Ph.D.  
Medical Department  
3M Company

# Determinants of Responses



# Physical and Chemical Properties

# Chemical and Physical Properties

- Perfluorinated alkyl acids (PFAAs)
  - Exceptionally stable
  - Non-reactive
  - Solubility varies
  - Amphiphilic “Organic” acids with low pKa
  - Essentially dissociated under most conditions
  - Surface active
  - Low Van der Waal's

# Physical/Chemical Determinants

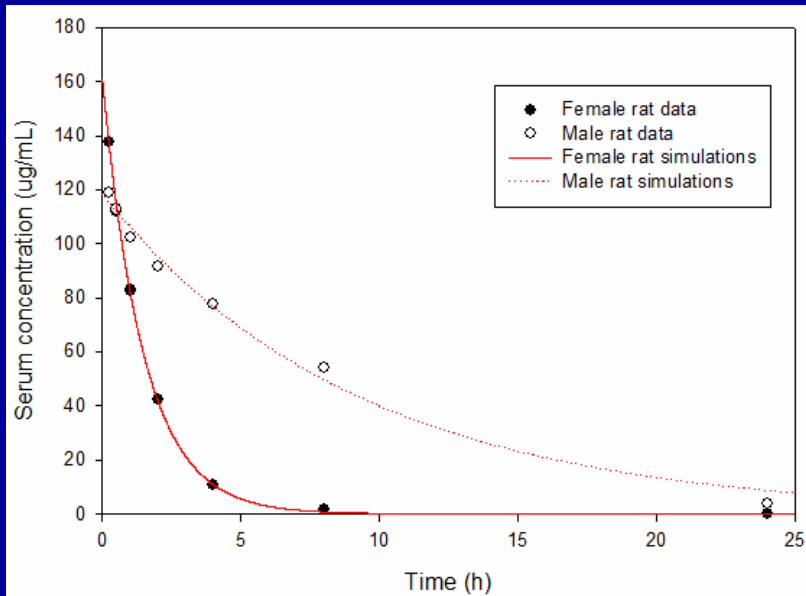
- Non-reactive
- Resemble free fatty acids (FFAs)
  - Do not enter into the biochemical reactions that use fatty acids as substrate.
- However, PFAAs may present as FFAs.
  - Transporters
  - Receptors
  - Carrier proteins
  - Membranes

# Pharmacokinetics

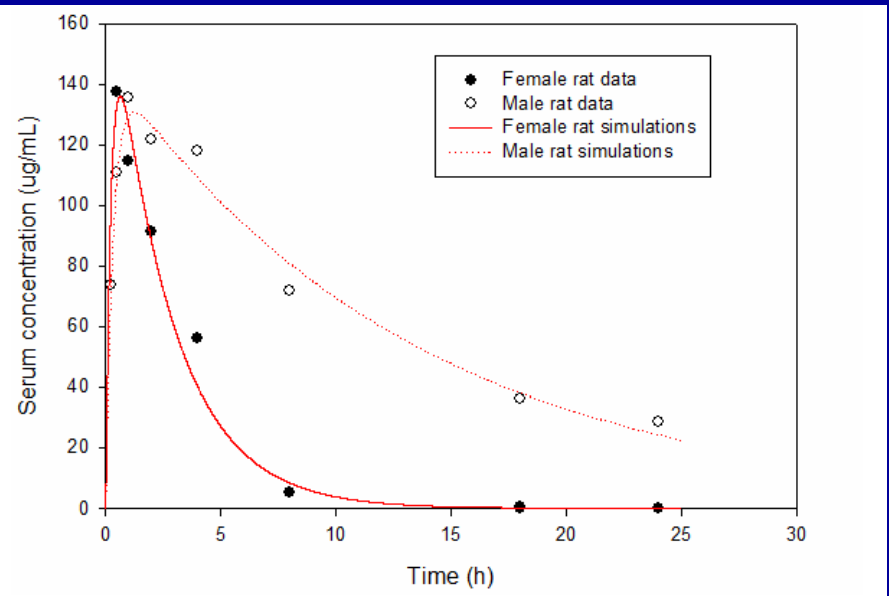
# Pharmacokinetics - Absorption

- Oral absorption is efficient for PFAAs

30 mg NH<sub>4</sub><sup>+</sup> PFBA/kg iv



30 mg NH<sub>4</sub><sup>+</sup> PFBA/kg gavage



From: Chang et al. (2008) Toxicol Sci (Epub ahead of print).

# Pharmacokinetics - Distribution

- Distribution is predominantly extracellular
  - Volumes of distribution ~200 mL/kg BW
  - Primarily liver, plasma/serum, kidney<sup>1</sup>
- Liver uptake
  - Compound and species dependent
  - Dose dependent<sup>2</sup>
  - Membranes<sup>2</sup>
  - Cytoplasmic proteins<sup>3</sup>

<sup>1</sup>Vanden Heuvel et al. (1991) J Biochem Toxicol 6, 83-92.

<sup>2</sup>Kudo et al. (2007) Biol Pharm Bull 30, 1535-1540.

<sup>3</sup>Han et al. (2005) Drug Chem Toxicol 28, 197-209.

# Serum Concentration as an Index of Exposure

- For compounds that distribute with relatively low volumes of distribution and are highly bound to serum protein, serum concentration is a reliable index of exposure that can be related to health outcomes.

# Pharmacokinetics - Metabolism

- No known metabolic pathways in mammalian species
- Prohibitive chemical properties for metabolism

# Pharmacokinetics - Excretion

- Complex, dependent on
  - Carbon number
  - Sulfonates versus carboxylates
  - Branched versus linear chain
  - Species
  - Sex
- Urinary and biliary elimination pathways
- Enterohepatic circulation
- Determines residence time in body

# Pharmacokinetics – Excretion

## Does Size Really Matter?

- Carbon number (size) may influence rate of elimination (urine vs. enterohepatic?)
- Potential for decreased elimination with
  - Carboxylates with  $\geq 7$  perfluorinated carbons
  - Sulfonates  $\geq 6$  perfluorinated carbons

# Terminal $T_{0.5}$ of PF Carboxylates & Sulfonates in 3 Male and 3 Female Monkeys

PFAA	$C_x$	Mean Terminal $T_{0.5}$ (days)	
		Male	Female
PFBA <sup>1</sup>	$C_4$	1.7	1.7
PFHA <sup>2</sup>	$C_6$	1.5	0.81
PFOA <sup>3</sup>	$C_8$	21	33
PFBS <sup>2</sup>	$C_4$	4.0	3.5
PFHS <sup>2</sup>	$C_6$	141	87
PFOS <sup>2</sup>	$C_8$	132	110

<sup>1</sup>Chang et al. (2008) Toxicol Sci (Epub ahead of print).

<sup>2</sup>3M Company and Southern Research Institute, unpublished data.

<sup>3</sup>Butenhoff et al. (2004) Toxicol Sci 82, 394-406.

# Mean Human $T_{0.5}$ Values

PFAA	$C_x$	Mean $T_{0.5}$ (days)
PFBA <sup>1</sup>	$C_4$	3
PFOA <sup>2</sup>	$C_8$	1,378
PFBS <sup>3</sup>	$C_4$	30
PFHS <sup>2</sup>	$C_6$	3,109
PFOS <sup>2</sup>	$C_8$	1,976

<sup>1</sup>Chang et al. (2008) Toxicol Sci (Epub ahead of print).

<sup>2</sup>Olsen et al. (2007) Environ Health Perspect 115, 1298-1305.

<sup>3</sup>3M Company, unpublished data

# Serum Elimination Half-Lives of Perfluoroalkylsulfonates in Rats and Mice

PFAS	Serum Elimination Half-Life (d)	
	Rats	Mice
PFBS	0.17 - 0.19 <sup>a</sup>	
PFHxS	16 <sup>b</sup> - 29 <sup>c</sup>	Study Planned
PFOS	30 - 40 <sup>b,d</sup>	30 - 40 <sup>d</sup>

No major sex differences in elimination noted.

<sup>a</sup> Olsen et al. (2009) Toxicology 256, 65-74

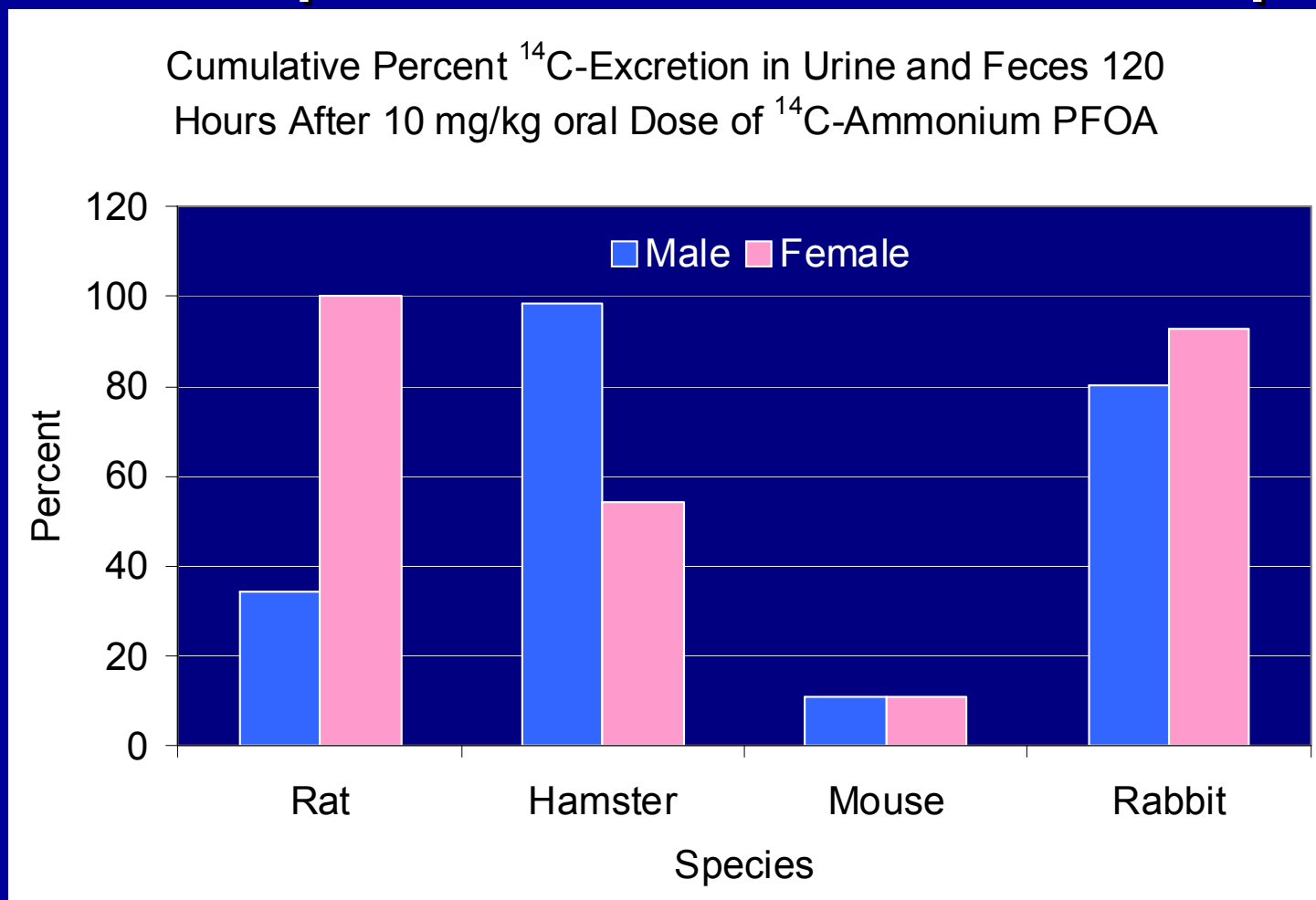
<sup>b</sup> Benskin et al. (2009) Environ Toxicol Chem 28, 542-554

<sup>c</sup> Sundström et al. (2009) DIOXIN 2009

<sup>d</sup> 3M preliminary data: formal PK analyses pending

# Pharmacokinetics – Excretion

## Is Sex Important? PFOA Example



Data from: Hundley et al. (2007) Drug Chem Toxicol 29, 137-145.

# Pharmacokinetics – Excretion

## Does Form Make a Difference?

- Branched PFOA eliminated from serum more rapidly than linear in monkeys<sup>1</sup> and humans<sup>2</sup>.
- Less branched than linear PFOA in serum at equivalent doses in rodents.<sup>3</sup>
- Branched PFBA eliminated faster than linear.
- Some differences also seen with PFOS

<sup>1</sup> Butenhoff *et al.*, unpublished

<sup>2</sup> Olsen *et al.*, unpublished

<sup>3</sup> Loveless *et al.* (2006) *Toxicology* 220, 203-217.

# Elimination of Branched Isomers of PFOS in Rats

De Silva et al. (2009)  
Environ Toxicol  
Chem 28, 555-567)

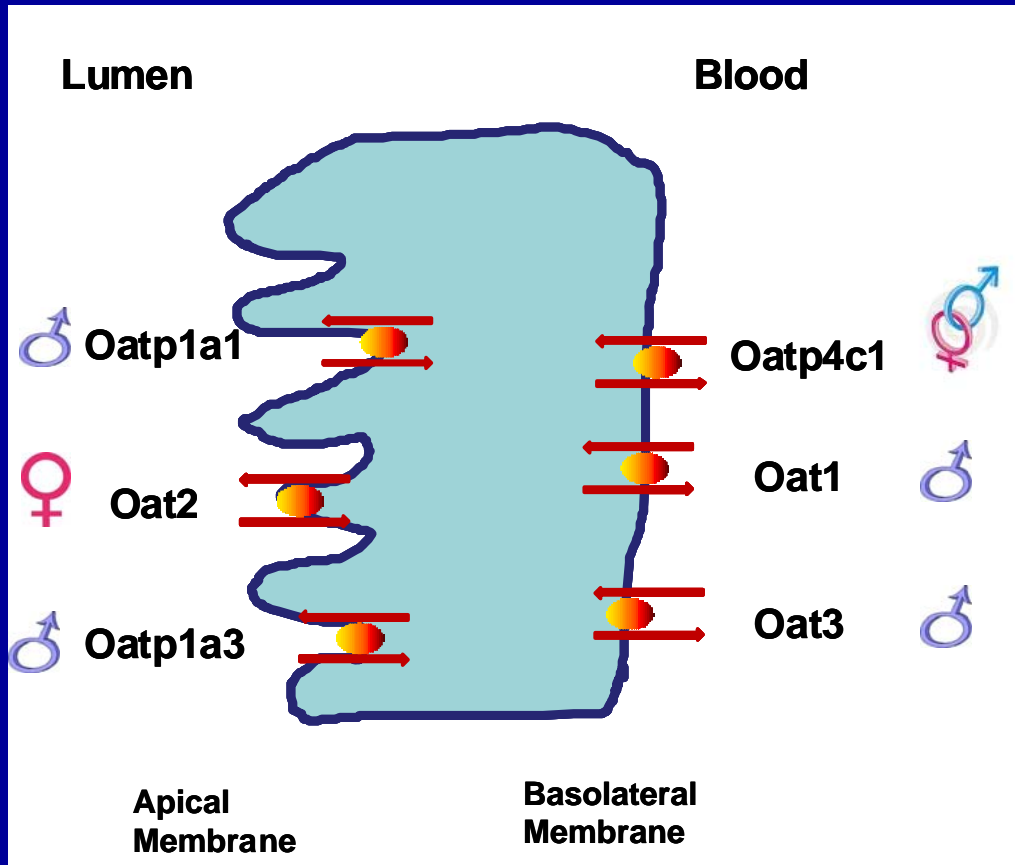
Table 2. Growth-corrected elimination rate constants ( $k_d$ ) and elimination half-lives ( $t_{1/2}$ ) with 95% confidence interval (CI) for perfluorooctane sulfonate (PFOS) isomers in male and female rat blood

Perfluorooctane sulfonate isomer	Mean $k_d$ ( $d^{-1}$ )	$k_d$ ( $d^{-1}$ ) (95% CI)	$t_{1/2}$ (d) (95% CI)	$r^2$
<b>Males</b>				
<i>n</i> -PFOS	0.0085	0.0065–0.010	82 (66–107)	0.92
<i>iso</i> -PFOS	0.011	0.0065–0.015	65 (47–107)	0.84
<i>5m</i> -PFOS	0.013	0.010–0.016	55 (44–72)	0.93
<i>4m</i> -PFOS	0.015	0.010–0.021	45 (34–68)	0.88
<i>3m</i> -PFOS	0.011	0.0083–0.014	62 (49–84)	0.92
<i>1m</i> -PFOS	0.0067	0.0024–0.011	103 (63–288)	0.66
<i>t</i> -butyl-PFOS	0.023	0.016–0.030	30 (23–43)	0.90
B <sub>7</sub> -PFOS	0.013	0.010–0.016	54 (44–69)	0.94
B <sub>8</sub> -PFOS	0.021	0.0054–0.036	33 (19–130)	0.85
<b>Females</b>				
<i>n</i> -PFOS	0.0087	0.0073–0.0095	83 (73–95)	0.98
<i>iso</i> -PFOS	0.0181	0.0085–0.028	38 (25–81)	0.75
<i>5m</i> -PFOS	0.0242	0.014–0.034	29 (20–49)	0.79
<i>4m</i> -PFOS	0.0284	0.019–0.038	24 (18–36)	0.89
<i>3m</i> -PFOS	0.0215	0.0098–0.033	32 (21–71)	0.76
<i>1m</i> -PFOS	0.0012	0.0089–0.0112	590 (62–78)	0.028
<i>t</i> -butyl-PFOS	0.0919	0.057–0.13	7.5 (5–12)	0.85
B <sub>7</sub> -PFOS	0.0143	0.0052–0.025	46 (28–133)	0.64

# Pharmacokinetics - Excretion

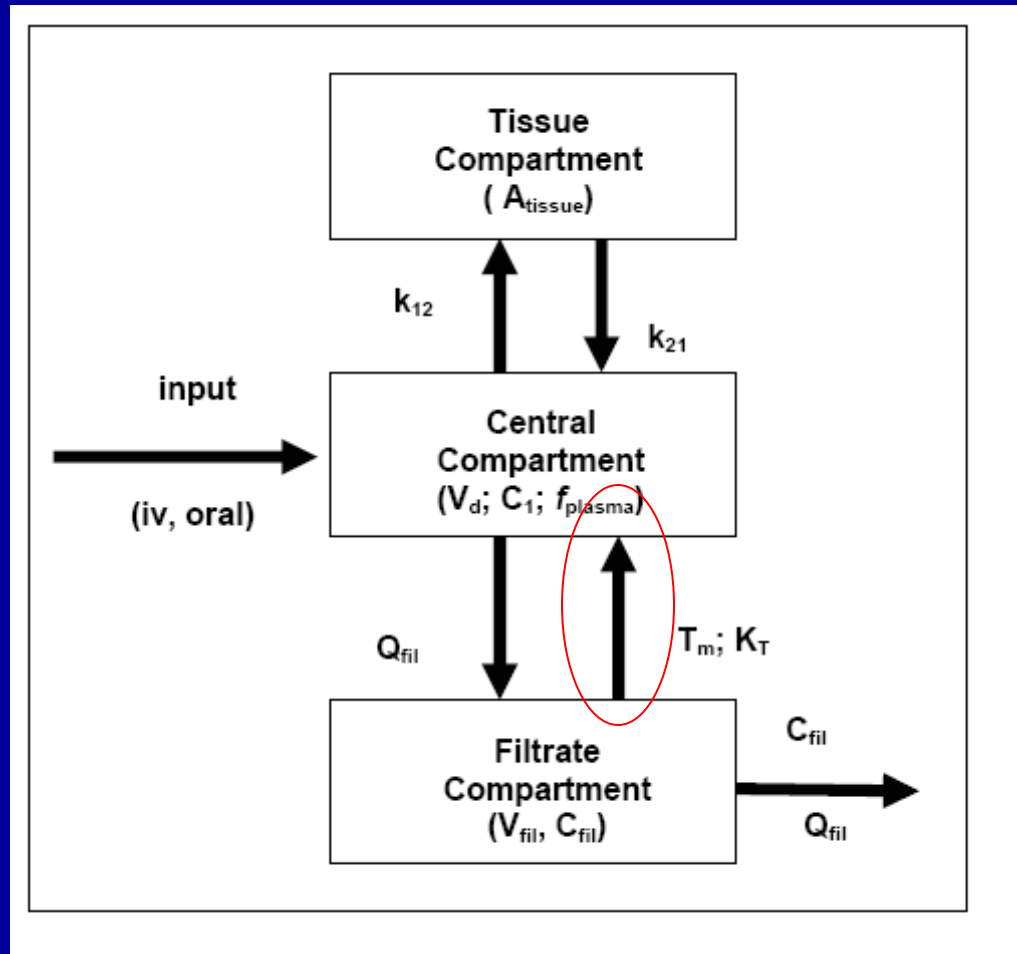
- Active renal proximal tubular reabsorption
- First suggested by Kudo et al. (2002)
  - Based on increased mRNA for Oatp1 in male rats
- First modeled by Andersen et al. (2006)
  - Cynomolgus monkey PK data for PFOA and PFOS fit reabsorption model
- Evidence in rat by Katakura et al. (2007)
  - Oat3 and Oatp1 may be reabsorption transporters

# Uptake transporters in renal proximal tubule cells



Based on subcellular localization, Oat1 and Oat3 may be responsible for active renal secretion of PFHA, PFOA and PFNA while Oatp1a1 may be responsible for reabsorption of PFDA, PFNA and PFOA. (From poster by Weaver and Hagenbuch, 2008).

# A Schematic for a Physiologically-Motivated Renal Resorption Pharmacokinetic Model<sup>1</sup>



<sup>1</sup> Andersen *et al.* (2006) Toxicology (in press)

# Biological Interactions

# Biological Interactions of PFAs

- Resemblance to free fatty acids (FFA)
  - But not metabolized
- Expected interactions
  - Biological membranes
  - Organic anion transport processes
  - Protein ionic binding sites
  - Activation of biochemical processes

# Biological Interactions - Membranes

- High concentration effects
- Membrane fluidity (surfactant effect)
  - Incorporation (PFOS > PFOA >> OS)<sup>1</sup>
  - PFOA and PFOS<sup>1,2</sup>, not PFHS and PFBS<sup>2</sup>
- Gap Junction Intercellular Communication<sup>3,4</sup>
- PFOS & PFOA - slight surfactant effect on mitochondrial membranes *in vitro*<sup>5</sup>

<sup>1</sup>Xie et al. (2007) Biochim Biophys Acta 1768, 1299-1308.

<sup>2</sup>Hu et al. (2003) Comp Biochem Physiol C Toxicol Pharmacol 135, 77-88.

<sup>3</sup>Upham et al. (1998) Int J Cancer 178, 491-495.

<sup>4</sup>Hu et al. (2002) Toxicol Sci 68, 429-436.

<sup>5</sup>Starkov and Wallace (2002) Toxicol Sci 66, 244-252.

# Biological Interactions – Organic Anion Transport

- Importance in uptake and elimination
  - Perfluoroalkyls
  - Other organic anions
- Induction of transporters
- Competition with endogenous substrates

# Biological Interactions - Protein Ionic Binding

- Albumin
  - Major carrier protein in serum<sup>1,2,3</sup>
  - Saturable<sup>1</sup>
  - Competition with endogenous substrates
    - Steroid hormones<sup>1</sup>
    - Thyroid hormones<sup>4</sup>
  - Carbon number (size) and solubility

<sup>1</sup>Jones et al. (2003) Environ Toxicol Chem 22, 2639-2649.

<sup>2</sup>Han et al. (2003) Chem Res Toxicol 16, 775-781.

<sup>3</sup>3M and Southern Research Institute, unpublished report, USEPA Docket AR-226.

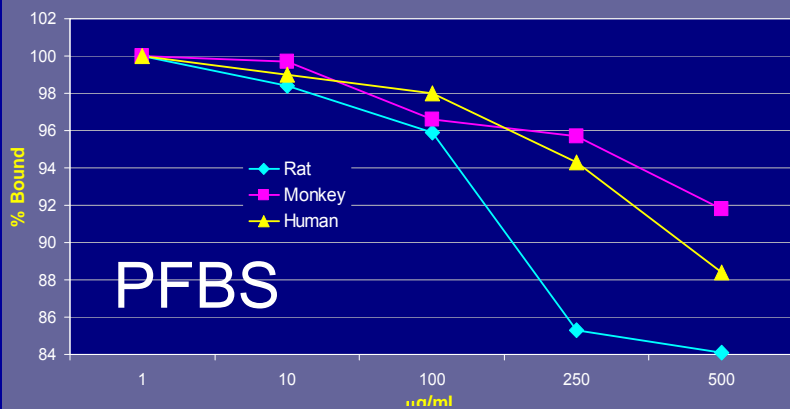
<sup>4</sup>Chang et al (2008) Toxicology 243, 330-339.

# Serum Protein Binding

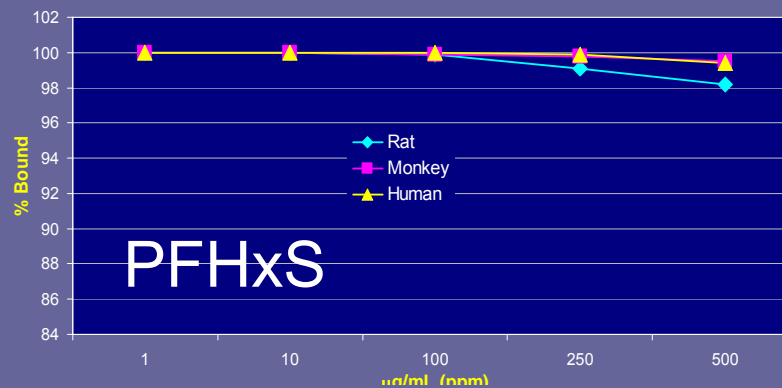
- At relevant concentrations, PFBS, PFHxS, PFOS, and PFOA are highly bound in rat, monkey, and human serum.
- Binding to serum proteins limits bioavailability.
- Binding capacity and/or affinity appears less in rat for all compounds.

# Percent PFBS, PFHxS, PFOS, and PFOA Bound in Rat, Monkey, and Human Sera from 1,000 – 500,000 ng/mL

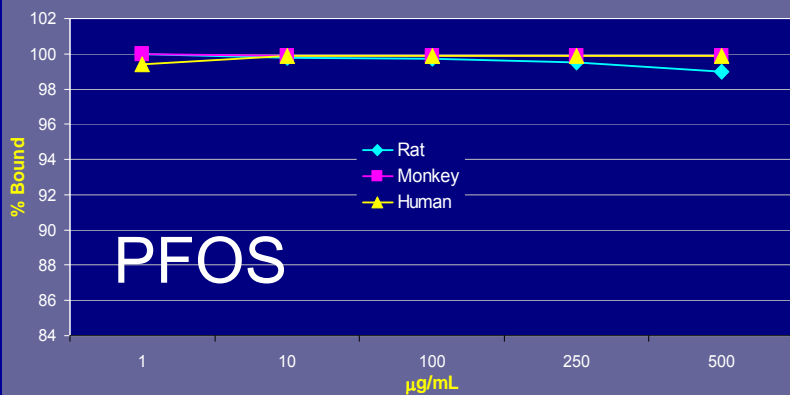
PFBS Serum Protein Binding in Rats, Monkeys, and Human Serum Over a Concentration Range of 1 - 500 ug/mL (ppm)



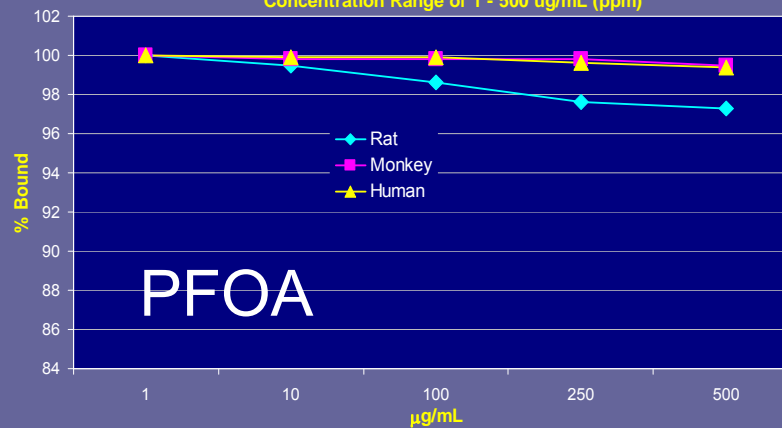
PFHxS Serum Protein Binding in Rat, Monkey, and Human Serum Over a Concentration Range of 1 - 500 ug/mL (ppm)



PFOS Serum Protein Binding in Rat, Monkey, and Human Serum Over a Concentration Range of 1 - 500 ug/mL (ppm)



PFOA Serum Protein Binding in Rats, Monkeys, and Human Serum Over A Concentration Range of 1 - 500 ug/mL (ppm)



# Percent Binding of PFBS, PFHxS, PFOS, and PFOA to Isolated Human Serum Protein Fractions (in vitro)

Percent of Perfluoroalkyl Bound				
	PFBS	PFHxS	PFOS	PFOA

Albumin	93.5	>99.9	99.8	99.7
$\beta$ -Lipoprotein	<0.1	64.1	95.6	39.6

- Proteins added to buffer at physiological concentration.
- Perfluoroalkyls incubated with proteins at 10,000 ng/mL concentration

3M Company and Southern Research Institute, unpublished data

# Competition with Thyroid Hormone

- Competitive displacement of T3 and T4 from binding sites
  - Similar to FFA, salicylates, other PPAR $\alpha$  agonists
- Increased metabolism and elimination of TH
- Hypothyroxinemia with maintenance of adequate free hormone
  - TSH not clinically increased
  - Must use equilibrium dialysis or ultrafiltration reference methods to obtain accurate free hormone concentration (negative bias)
  - Free hormone levels maintained or transiently increased
- H-P-T axis unaffected
- No effect on thyroid structure

# Thyroid Hormones Binding in Serum

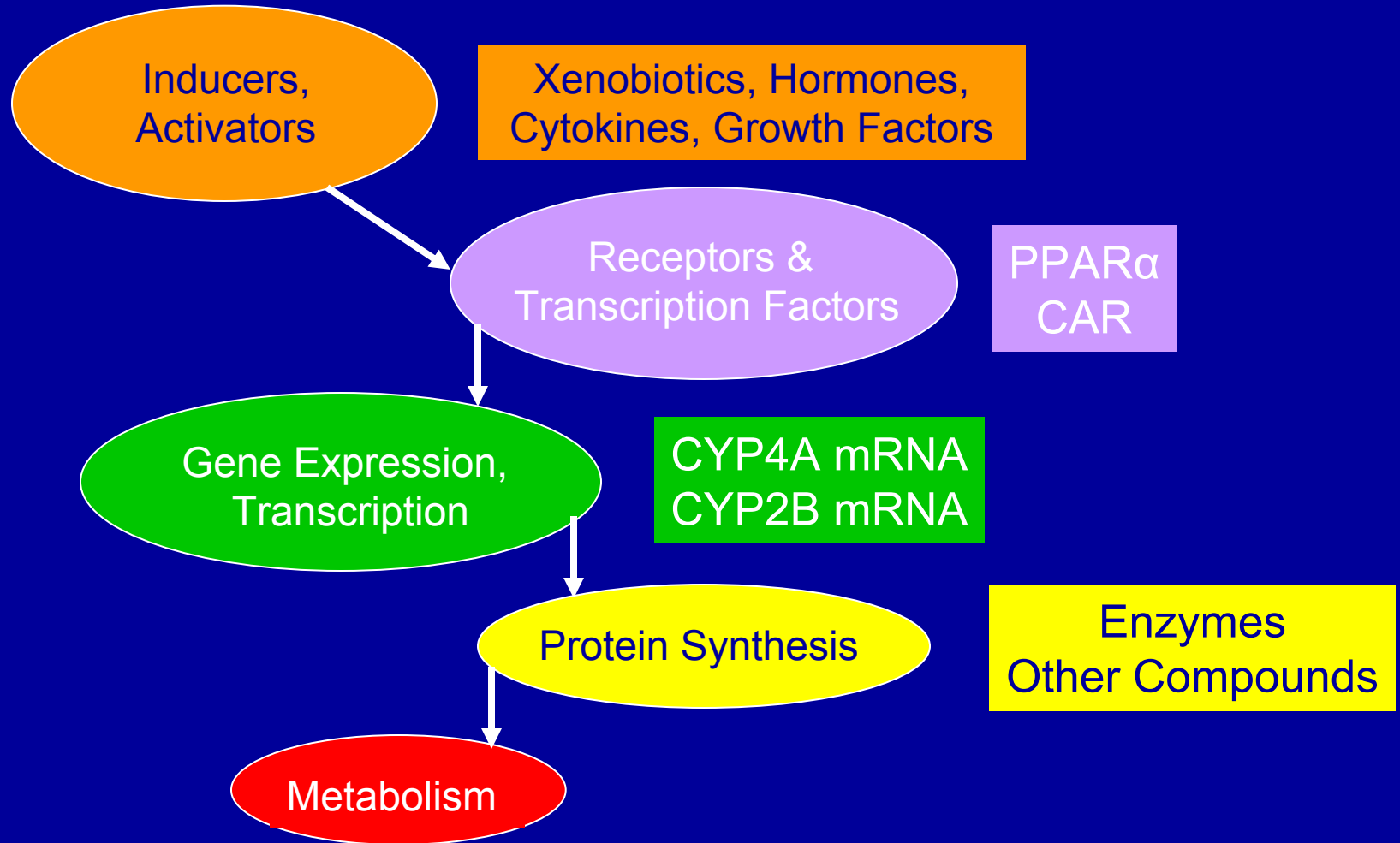
		Thyroid Hormone Binding Protein								
		Albumin			Transthyretin			Thyroid Binding Globulin		
Species	T1/2 of T4 (d)	% Bound	Affinity to T3	Affinity to T4	% Bound	Affinity to T3	Affinity to T4	% Bound	Affinity to T3	Affinity to T4
Rat	0.5 - 1	>90	2E5/M	1.5E6/M	---	---	---	---	---	---
Human	5 - 9	5	2E5/M	1.5E6/M	20	1E6/M	2E8/M	75	1E9/M	1.1E10/M

- >90% of TH bound to albumin in rats
- 75% of TH on TBG in humans
  - Only 5% on albumin; 20% on transthyretin
- TBG has orders of magnitude greater affinity
- Increased metabolism and excretion of hormone (via CAR & PPAR $\alpha$  activation)
- Leads to widely increased sensitivity in rat

# Rats More Sensitive to Thyroid Endpoints than Humans

- Displacement of loosely bound hormone
  - A much smaller proportion of human thyroid hormone is displaceable.
- Increased metabolism of hormone
  - Humans are much less sensitive to PPAR $\alpha$  activation and metabolism is generally slower.
- The differences between rats and humans are widely recognized.

# Activation of Molecular Processes



Based on: Waxman (1999) Arch. Biochem. Biophys. 369, 11-23.

# Some Common Nuclear Receptors Controlling CYP Induction

<u>Receptor</u>	<u>Typical Activator</u>
• PPAR $\alpha$	Fatty acids, Fibrates
• PPAR $\gamma$	Rosiglitazone
• CAR	Phenobarbital
• PXR	Steroids, Dexamethasone
• LXR $\alpha$	Cholesterol
• FXR	Bile acids
• RXR	Retinoic acid
• TR	Triiodothyronine

# Nuclear Receptor Activation

- Numerous studies in various *in vitro* and *in vivo* models
- Most studied perfluoroalkyls activate PPAR $\alpha$ 
  - Varied activity
  - Rat, mouse, and human isoforms
- PFOS and PFOA weak agonists for PPAR $\alpha$  and PPAR $\gamma$  compared to endogenous ligands or targeted drugs<sup>1</sup>
- Evidence for CAR and PXR agonism
  - PPAR $\alpha$  dominates

<sup>1</sup> Vanden Heuvel et al. (2006) *Toxicol Sci* 92, 476-489.

# Species Differences in PPAR $\alpha$

- Rodents and humans
  - Expression level of PPAR $\alpha$  (rodent >> human)
  - Hepatocellular hypertrophic response (rodent >> human)
- Human PPAR $\alpha$  not associated with hepatic hyperplasia

<sup>1</sup>Cheung et al. (2004) *Cancer Res* 64, 3849-3854.

<sup>2</sup>Morimura et al. (2006) *Carcinogenesis* 27, 1074-1080.

<sup>3</sup>Shah et al. (2007) *Mol Cell Biol* 27, 4238-4247.

<sup>4</sup>Yang et al. (2008) *Toxicol Sci* 101, 132-139.

# PPAR $\alpha$ Activation and PFOA

- mRNA transcripts from wild-type or PPAR $\alpha$ -null mice<sup>1</sup>
  - dietary administration of PFOA for 7 days
  - Most PFOA dependent genes regulated by PPAR $\alpha$
- Attributed responses in rodents
  - Hepatocellular hypertrophy (in part)
  - Hepatocellular tumors (in part)
  - Satiety
  - Immune system (in part)
  - Hypolipidemia
  - Most developmental effects
  - Increased fat metabolism

<sup>1</sup>Rosen et al. (2008) Toxicol Sci (Epub ahead of print).

# Reproductive and Developmental Results – Laboratory Animals

- No effect on functional aspects of reproduction
- Not selective teratogens
- Developmental delays noted in some cases
- Birth weight and weight gain affected in some cases
- Neonatal mortality with PFOS and PFOA

# Modes of Action - Current Thoughts

- Although *in utero* exposure of both PFOS and PFOA caused neonatal mortality, the adverse effects may be mediated by separate mechanisms
- PFOA likely acts through the PPAR $\alpha$  signaling pathway that regulates intermediary metabolism
- PFOS likely interacts with phospholipids of lung surfactant and interferes with lung inflation and pulmonary function

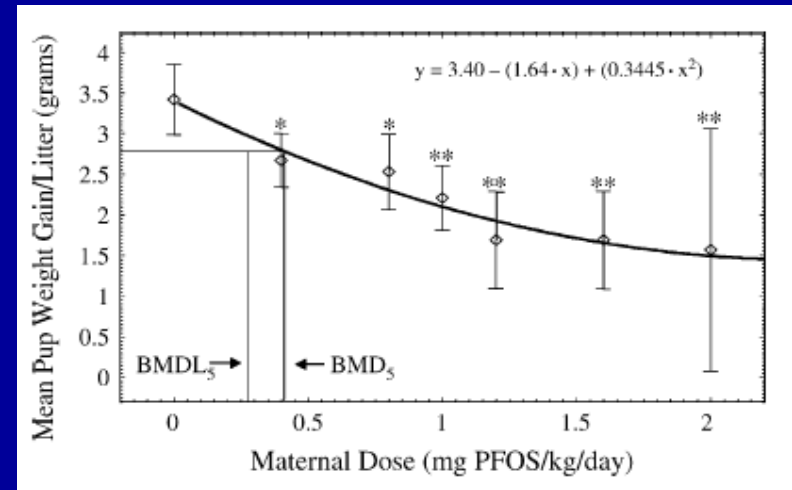
# Immune System – Laboratory Animals

- PFOS and PFOA
- Suppression of adaptive immunity in mice
- Enhancement of innate immunity in mice
- Attenuated by knocking out PPAR $\alpha$
- Species and strain differences
- Question as to whether effects noted are secondary to other changes (e.g. PPAR $\alpha$ -mediated liver effects)

# Body Weight – Laboratory Animals

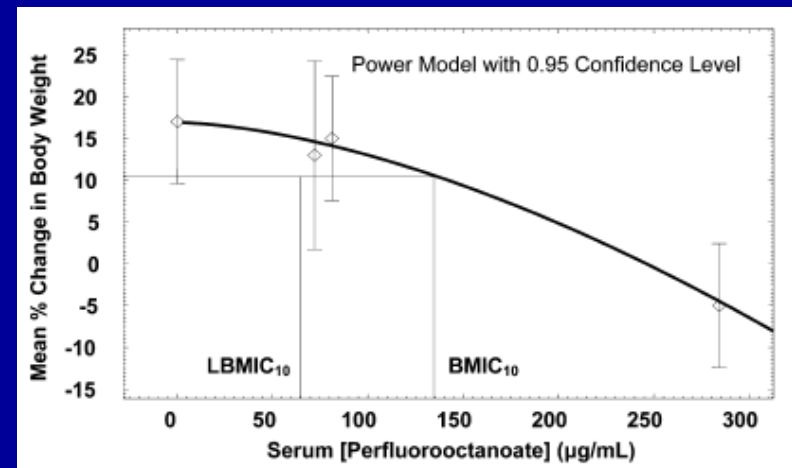
- Decreased weight gain in growing animals

Rat pups



- Weight loss at sufficient dose

Male monkeys



# Body Weight – Laboratory Animals

- Hypotheses
  - PPAR $\alpha$ -mediated oxidation of fat
  - Decreased appetite and food consumption

# In Summary

- Not possible to generalize about toxicity of Perfluoroalkyls
  - Similarities, yet significant differences
  - Quantitative or qualitative
  - Partially determined by
    - Pharmacokinetics
    - Physical and chemical properties
    - Potential biological molecular interactions
  - Species differences in response
  - Some endpoints in rodents may not be relevant in humans