Animal-Free Safety Assessment Education and Training Program

Covering Risk Assessment from start to finish

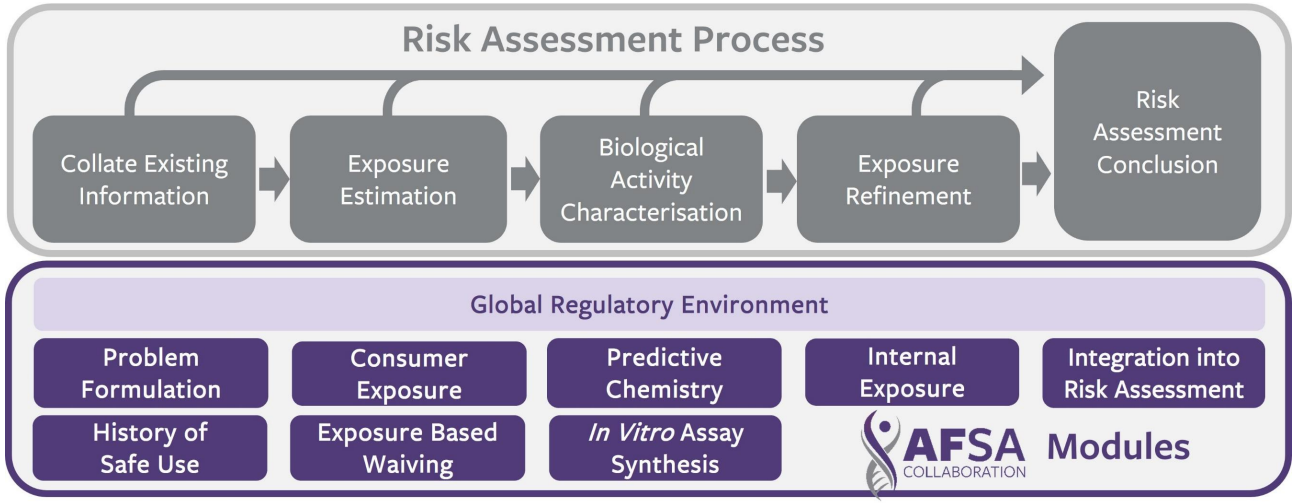
Dosimetry: internal exposure

5 May 2022
1:00 pm GMT/8:00 am EDT

Welcome and Introduction
Catherine Willett, Humane Society International

Dosimetry: internal exposure
Allison Schafer, Procter & Gamble
Rebecca Clewell, 21st century tox

Slido Quiz and Q&A



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Overview: AFSA Cosmetics Education and Training

Catherine Willett
Humane Society International

26 April 2022

The Animal-Free Safety Assessment Collaboration

The HSI-coordinated **Animal-Free Safety Assessment** (**AFSA**) **Collaboration** works to accelerate global adoption of a modern, species-relevant approach to safety assessment that will better protect people and our planet, and hasten the replacement of animal testing

COSMETICS

CHEMICALS

BIOLOGICALS

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COSMETICS

AFSA Education and Training in Next Generation Risk Assessment

CHEMICALS

BIOLOGICALS

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AFSA Cosmetics E&T

A Global Training Program in Non-Animal Risk Assessment

Scope

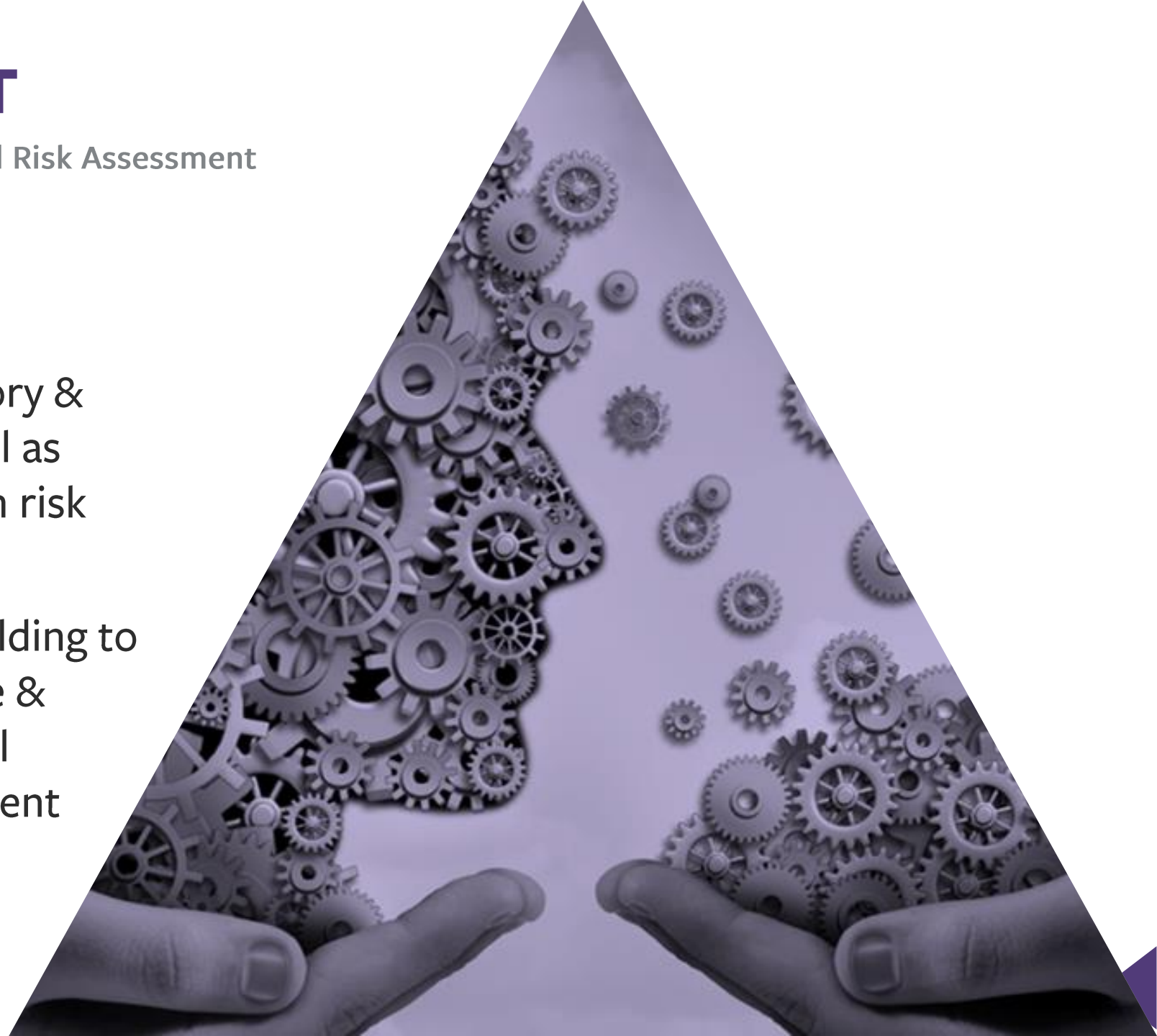
- Safety assessment of cosmetics and cosmetic ingredients without new animal data
- Covers all aspects of the process
  - Consumer exposure, external and internal
  - Acute local effects to systemic repeat effects
  - Information integration to make a risk decision
- Focus on *understanding* the information generated from the tools and *how to use* this information vs. how to perform or build the individual methods

AFSA Cosmetics E&T

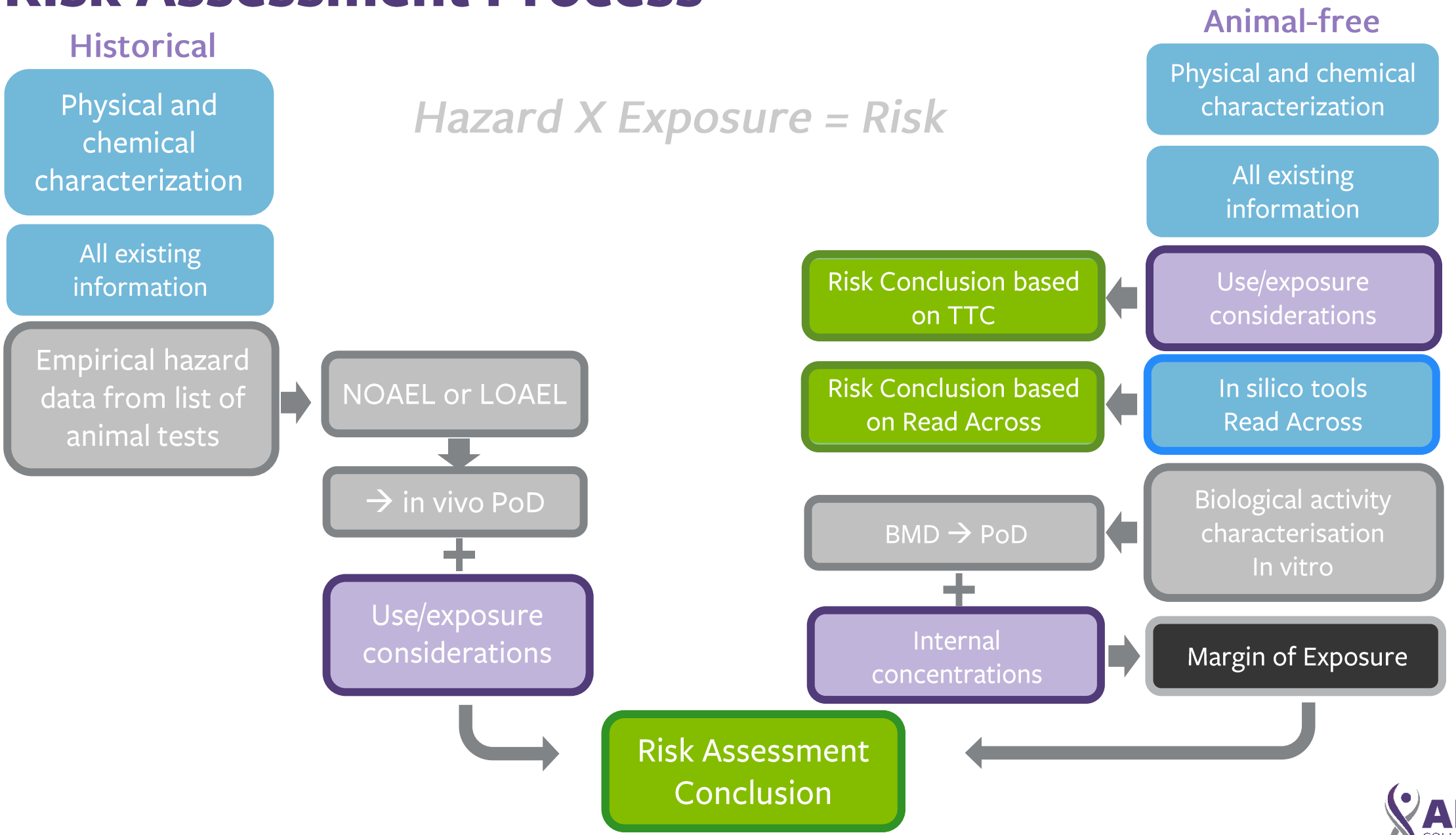
A Global Training Program in Non-Animal Risk Assessment

Purpose

- Address the needs of regulatory & regulated communities as well as other stakeholders involved in risk assessment of products
- Support regional capacity-building to achieve long-term acceptance & implementation of non-animal approaches to safety assessment

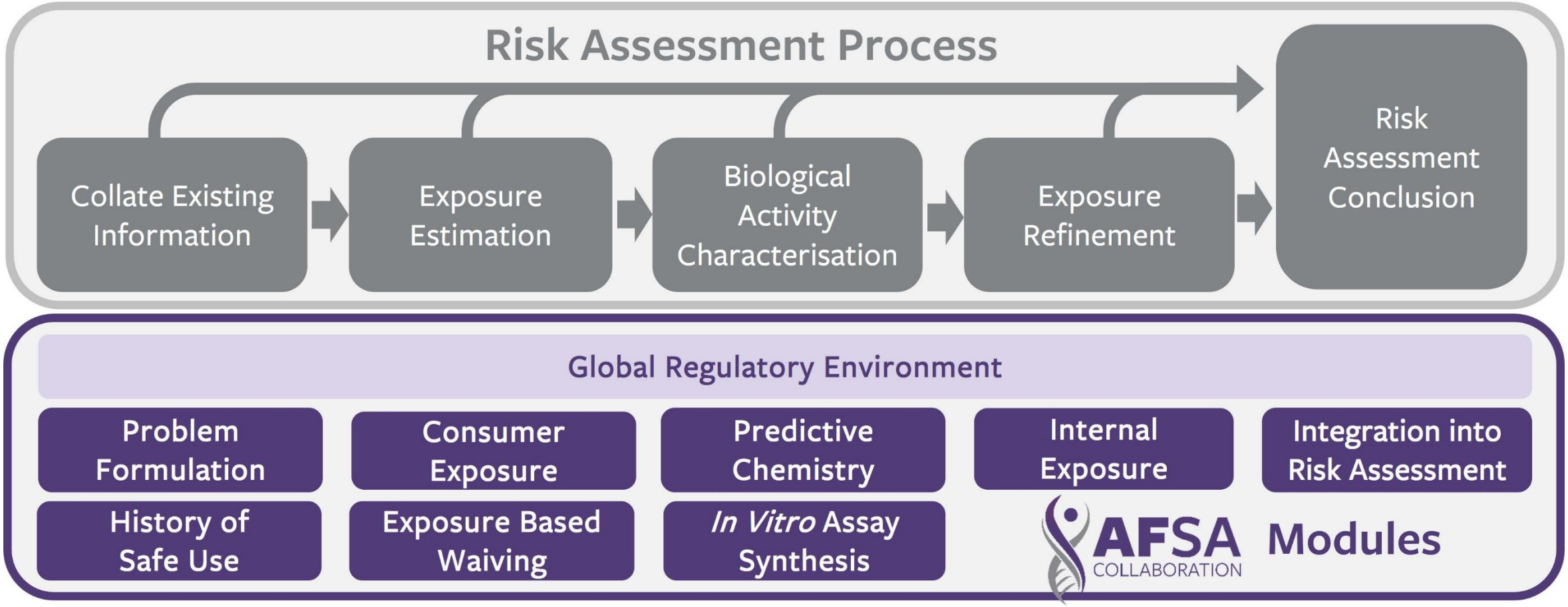


Risk Assessment Process

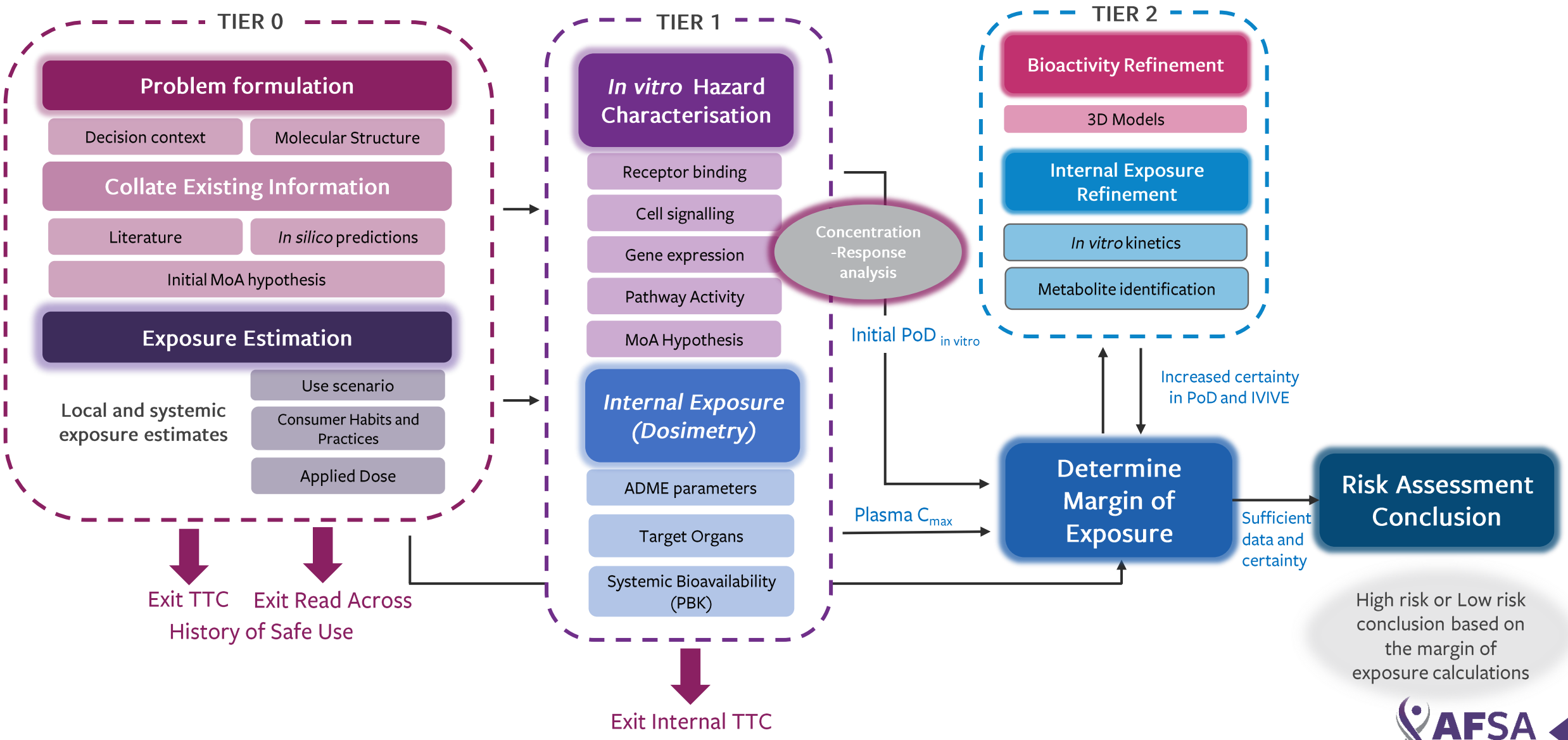


AFSA Cosmetics E&T

Covering Risk Assessment from start to finish



Next Generation Risk Assessment (NGRA) Framework



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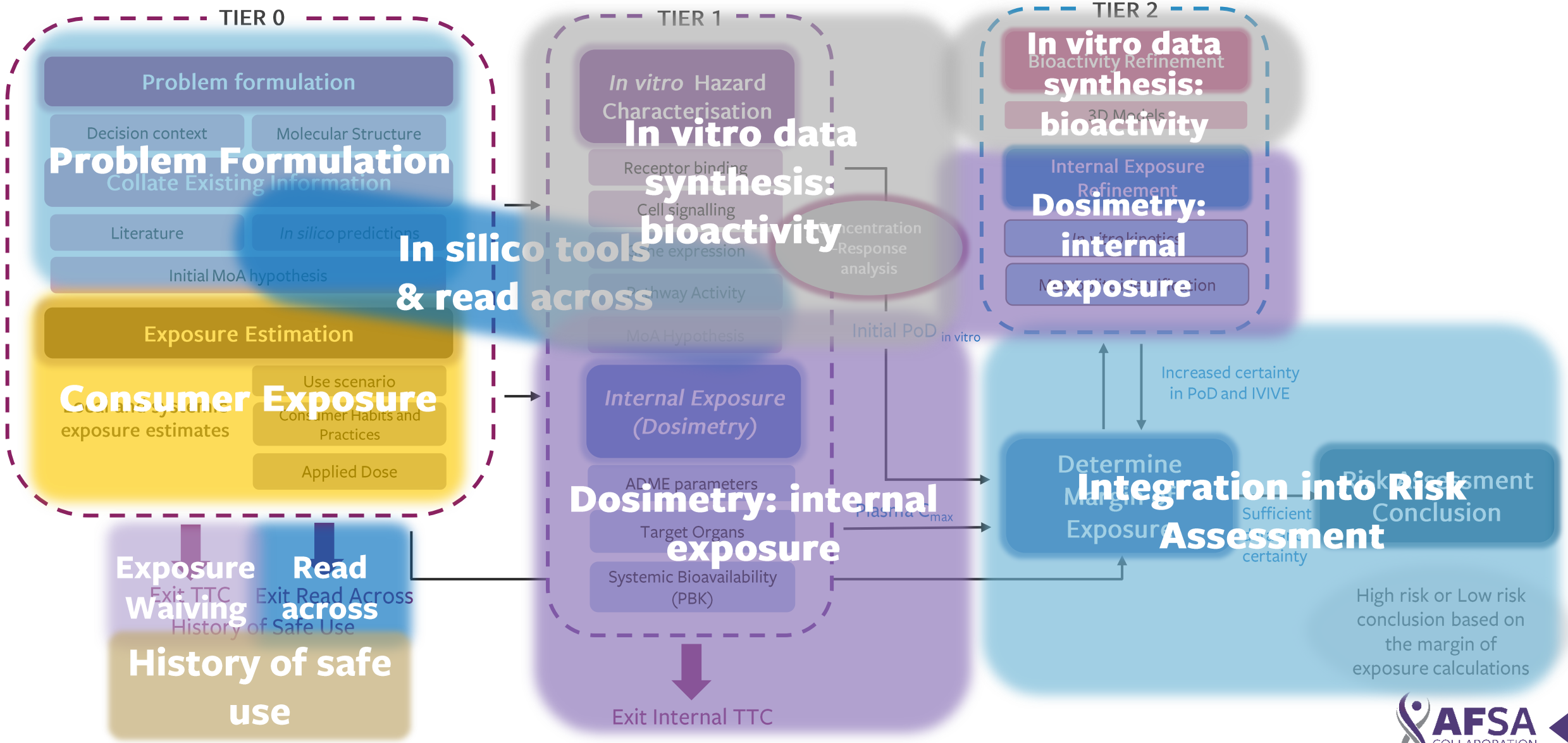
Next Generation Risk Assessment (NGRA) Framework



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Cosmetics Workstream Partners

HUMANE SOCIETY INTERNATIONAL

THE HUMANE SOCIETY OF THE UNITED STATES

Unilever

L'ORÉAL

P&G

Firmenich

AVON

iff

Givaudan

symrise

LUSH

Delphic HSE
SAFETY & REGULATORY SOLUTIONS

Lhasa Limited

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Renato de Ávila	Unilever
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Dosimetry: Internal Exposure

ALLISON SCHAFER, PROCTER & GAMBLE
REBECCA CLEWELL, 21ST CENTURY TOX

MAY 5, 2022

Overall Learning Objectives

Product Developers and evaluators

By the end of this webinar, you will be able to:

1. Describe the role of biokinetics in risk assessment
2. Outline four components of dosimetry (ADME)
3. List the applications of PBK modeling
4. List the types of compartments in PBK models
5. Outline the differences between IVIVE and PBK
6. Identify the applications of IVIVE

Introduction: Exposure and dosimetry in context of risk assessment

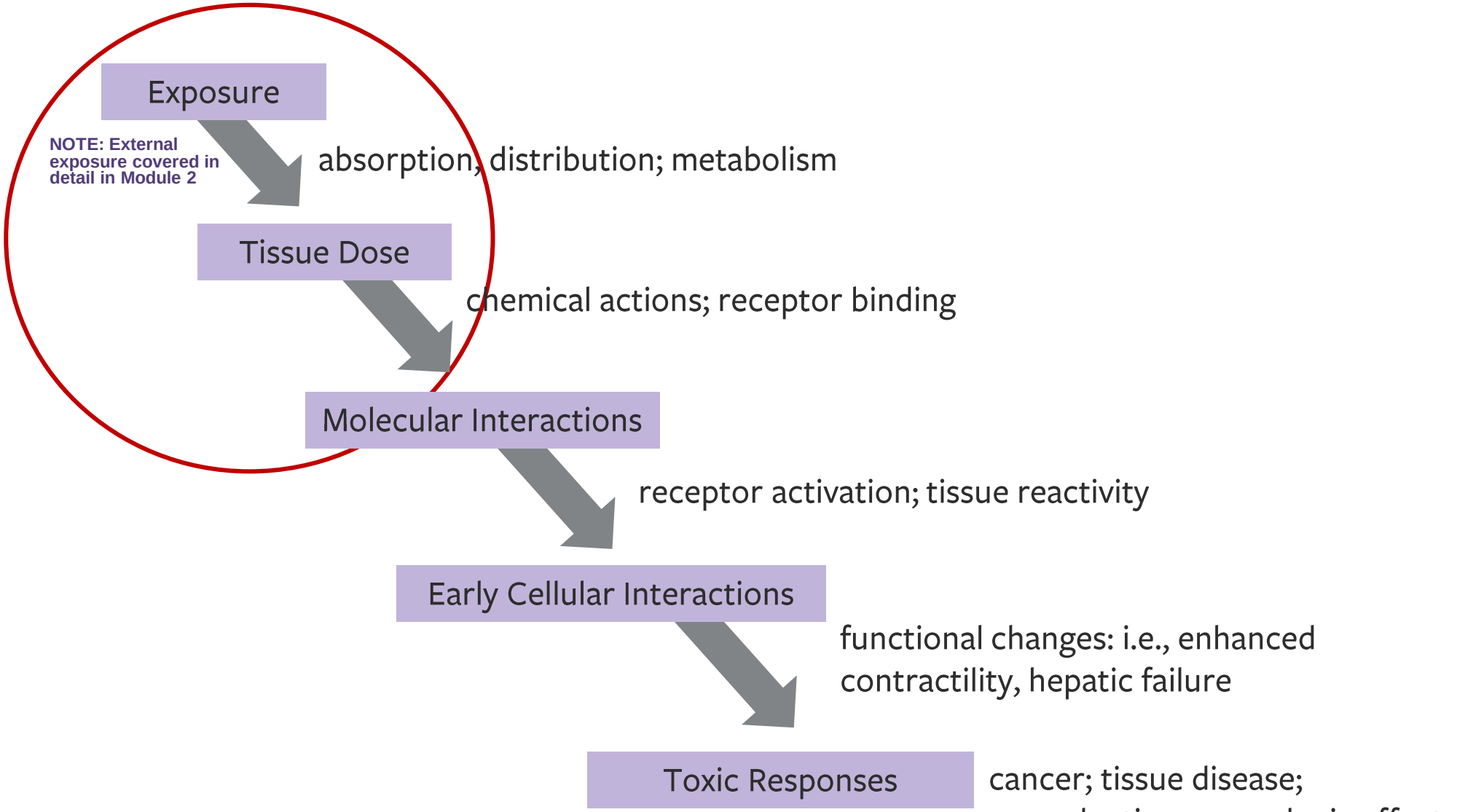
Why is dosimetry important?

- External exposure tells us the amount of a chemical that an individual may encounter but doesn't necessarily dictate the toxic response.
- Determining the internal exposure (i.e., the amount of the active form of the chemical that reaches the target tissue) provides a much more accurate prediction of the toxic response.

How do we predict target tissue dose?

- Target tissue dose can be predicted using the principals of biokinetics, the quantitative evaluation of the chemical kinetic processes that determine chemical uptake, distribution and excretion from the body.
- The goal of biokinetics is to develop internal dose metrics to help predict tissue toxic response.

Source to outcome continuum

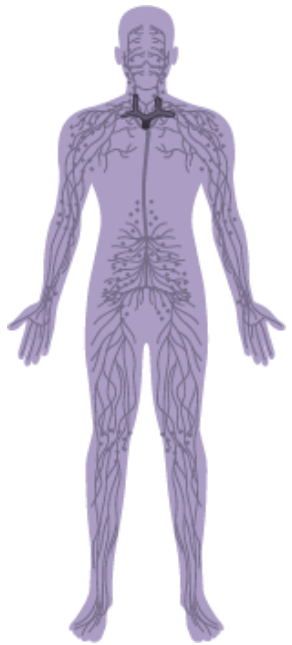


Exposure

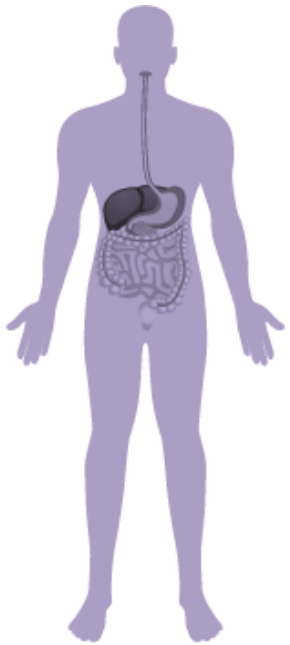
Defined as the measurement of both the amount of, and the frequency with which, a substance comes into contact with a person or the environment.

Routes of exposure:

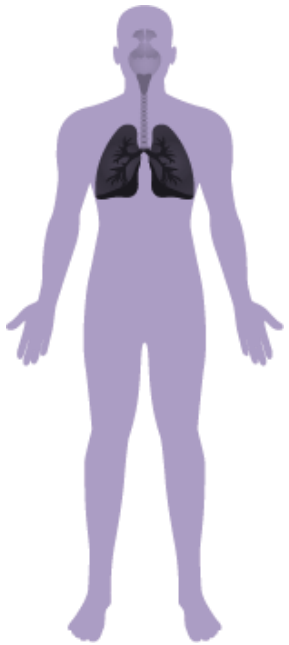
Skin (transdermal absorption)



Oral/GI tract (ingestion)



Lung (inhalation)



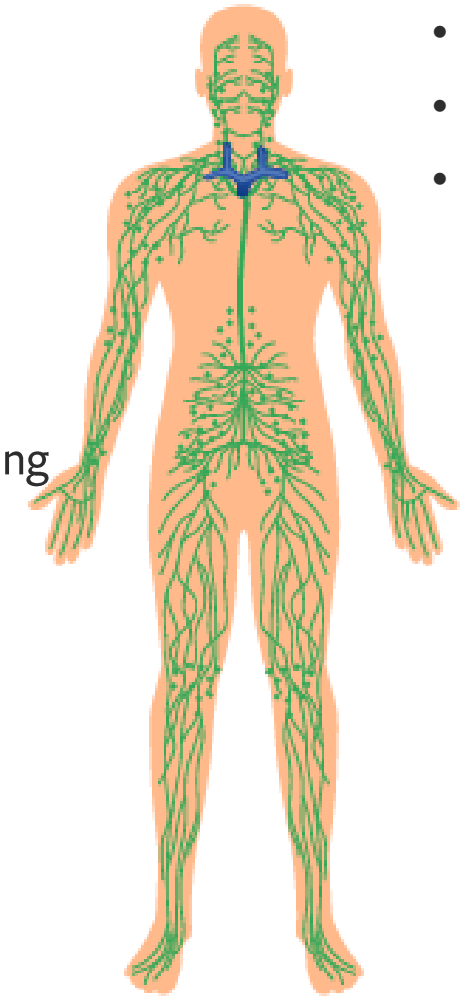
Other less common routes of human chemical exposure (usually associated with pharmaceuticals, or animal studies)

- Intravenous
  (injection/infusion into veins)
- Intramuscular
  (injection in muscle tissue)
- Subcutaneous
  (injection in subcutaneous fat)
- Intraperitoneal
  (injection into intraperitoneal cavity)

Use considerations: Routes of Exposure

Skin

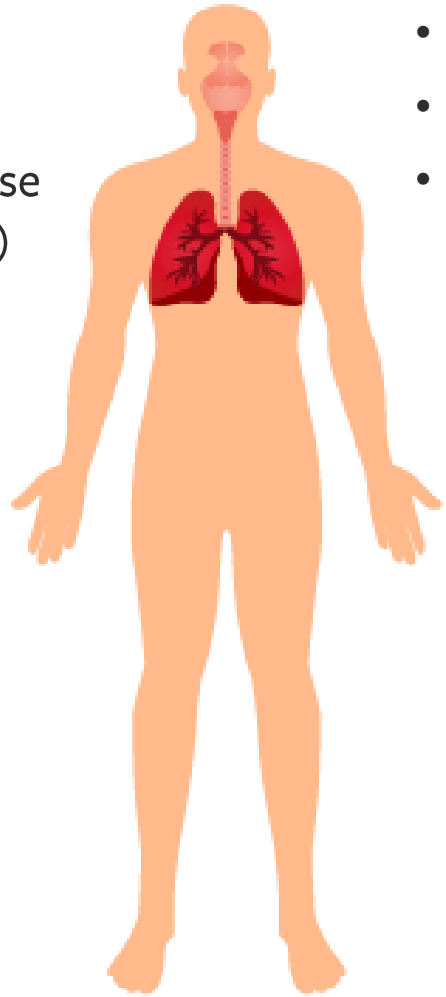
- Skin creams
- Deodorants
- Soap/cleansers
- Shampoo/ conditioner
- Shower gel
- Hand/dishwashing cleaners



Inhalation*

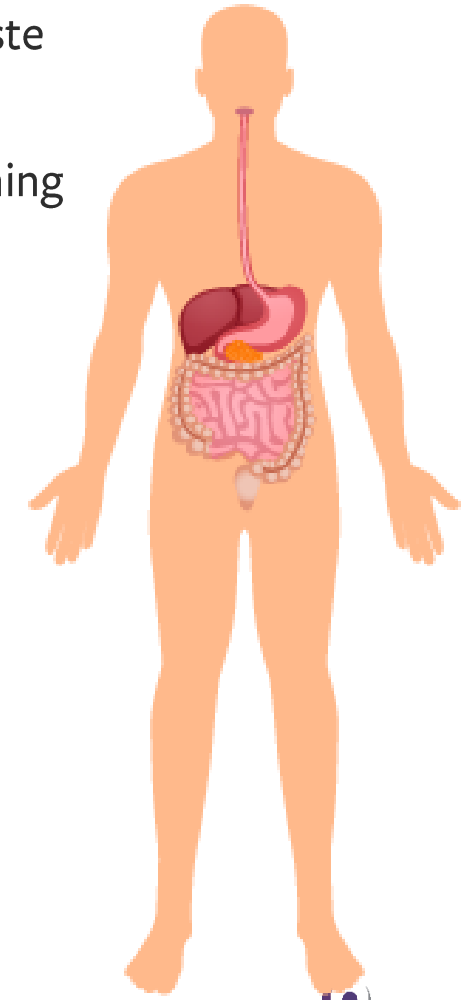
- Aerosols
- Pump sprays
- General purpose cleanser (GPC) trigger sprays

* Generally dependent on delivery system rather than product type.



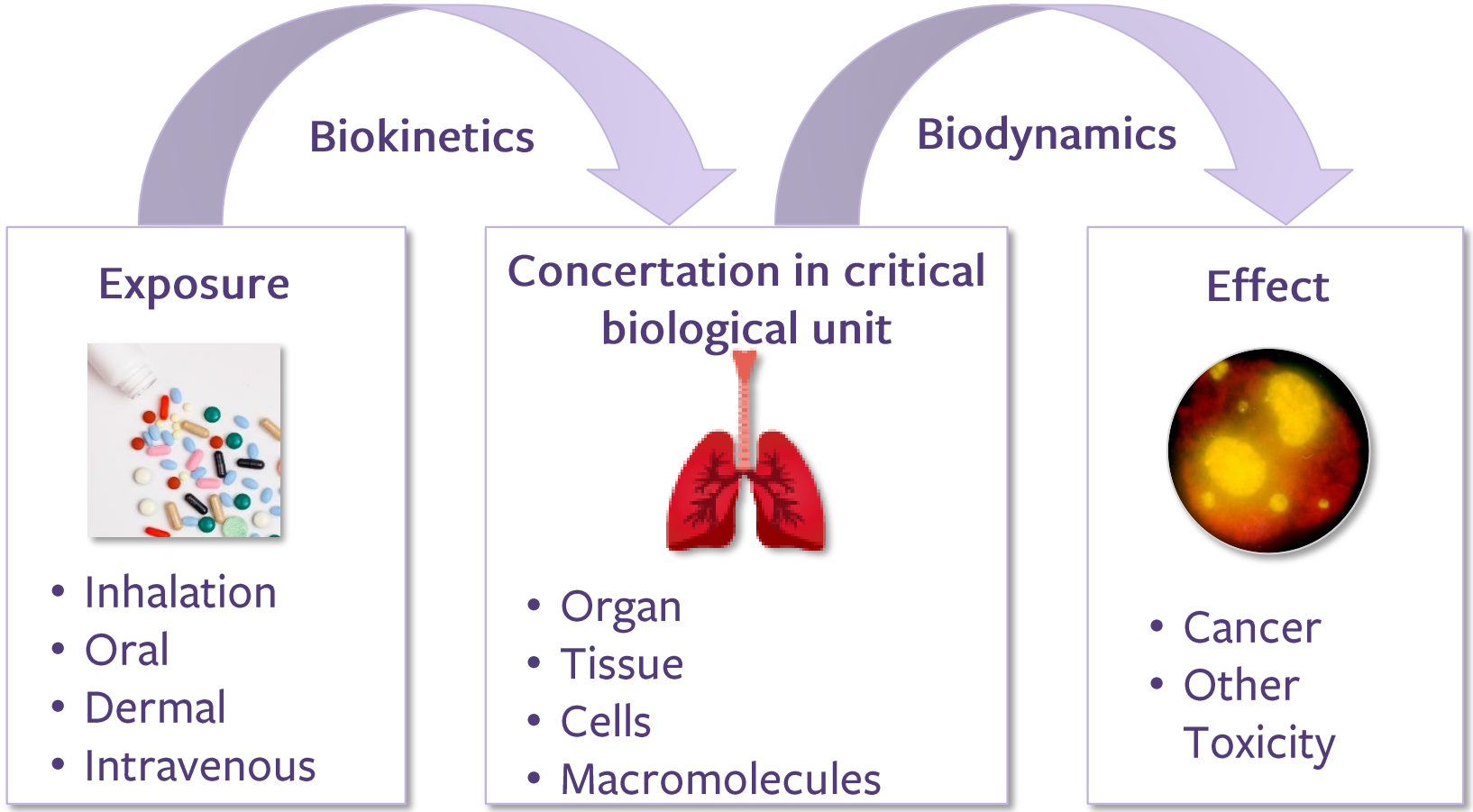
Ingestion

- Toothpaste
- Lipsticks
- Dishwashing residues



Biokinetics

The transport and metabolism of chemicals in a biological system



Tissue Concentration: the amount of chemical (in active form) that reaches the target tissue.

Point of Departure: the dose required for a particular tissue to have an effect

The goal of biokinetics is to determine the tissue dose associated with the point of departure.

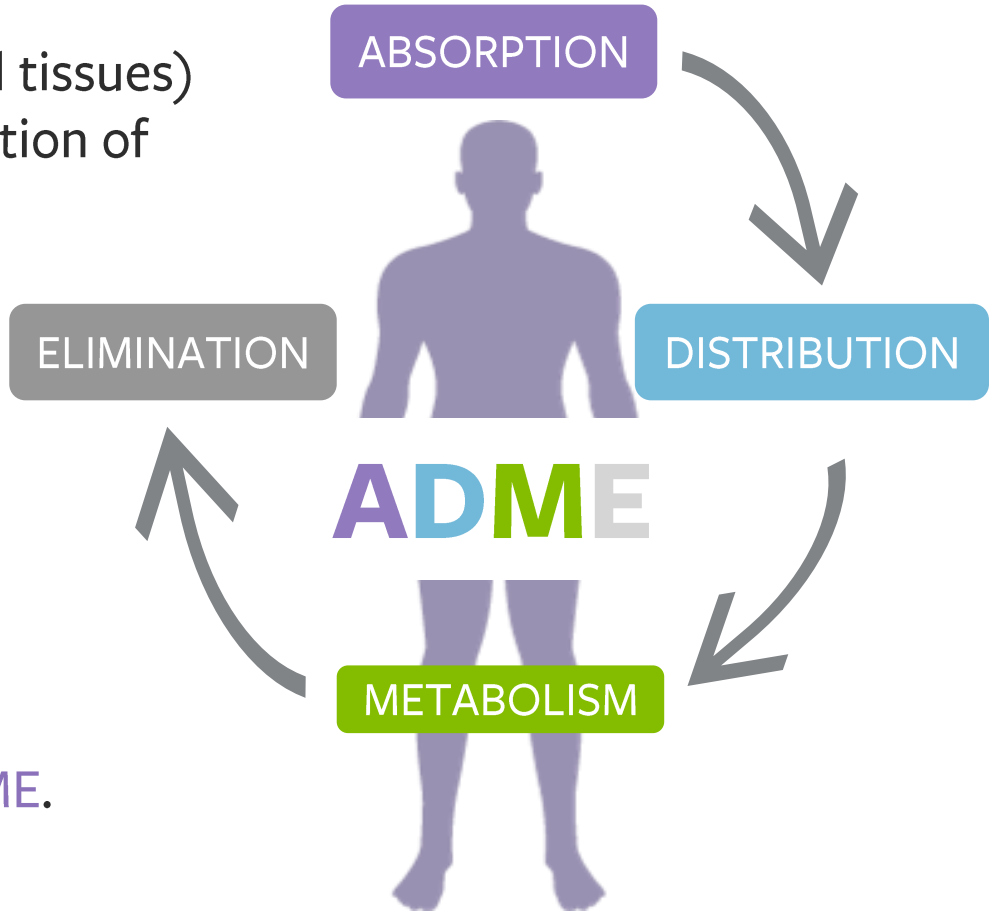
BIOKINETICS

Defining the basics

The four components of dosimetry

Dosimetry (chemical disposition; biokinetics):
Internal dosimetry (chemical concentration in the blood and tissues) is determined by the disposition of the chemical (or distribution of the chemical throughout the body).

- There are 4 main processes that determine chemical disposition and internal dosimetry:
    1. **Absorption**
    2. **Distribution**
    3. **Metabolism**
    4. **Elimination**
- Together, these 4 processes are often abbreviated as ADME.
- Chemical disposition (aka biokinetics) is a product of the composite actions of a chemical's ADME.



Components of Dosimetry: Absorption

Absorption: The uptake of chemicals into the body.

- Can occur through passive or active biochemical processes
- Sites of absorption: GI tract, lung, skin, plasma membrane (iv)

BIOAVAILABILITY

Describes how well the chemical is absorbed: the fraction of administered dose that enters systemic circulation

- Limitations in absorption through skin, GI or lung barriers can reduce bioavailability
- By definition, an intravenously administered dose is 100% bioavailable

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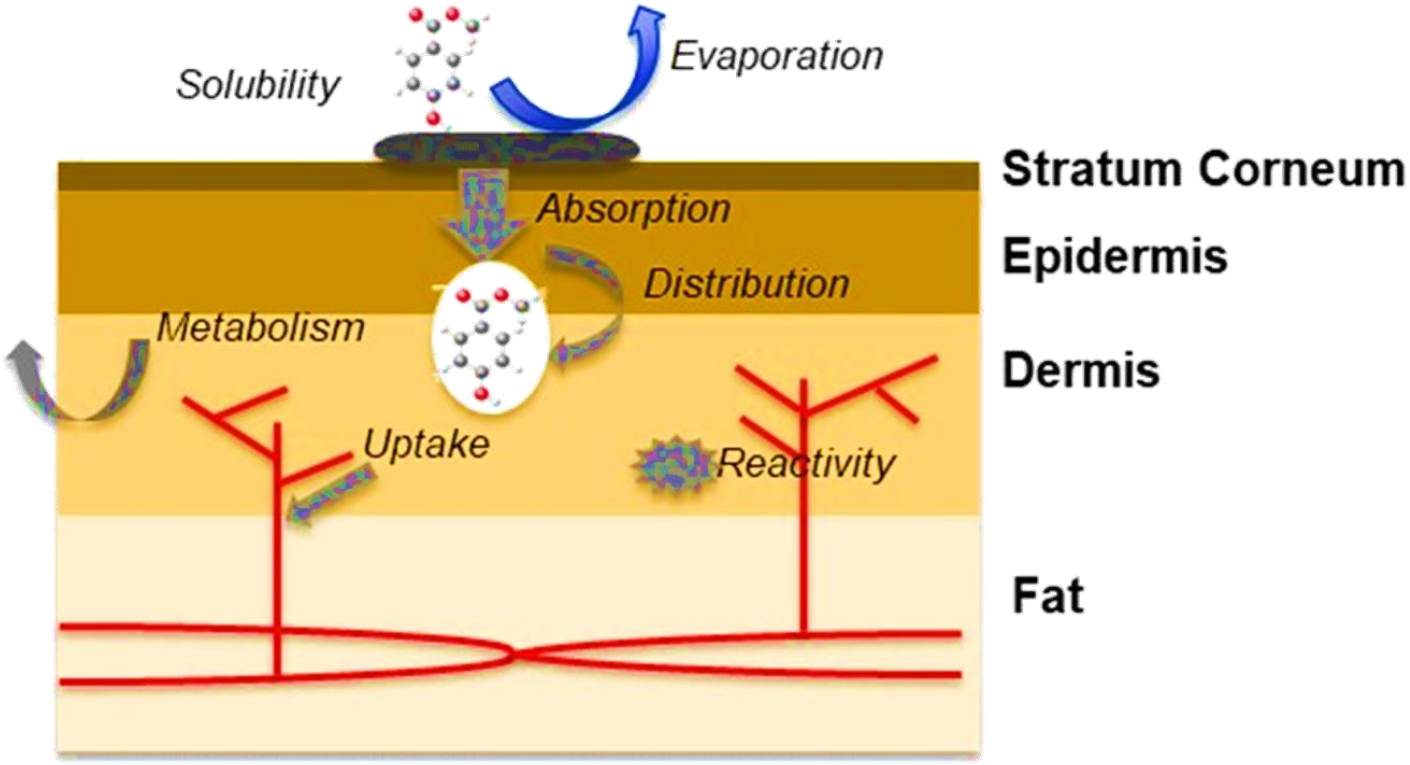
Absorption: Dermal Exposure

- Assumption of 100% skin penetration in the absence of data
- May be reduced by:
  - ✓ washing/wiping of skin
  - ✓ evaporation off of skin
  - ✓ metabolism in keratinocytes
  - ✓ distribution in skin

FACTORS INFLUENCING **SKIN BIOAVAILABILITY**

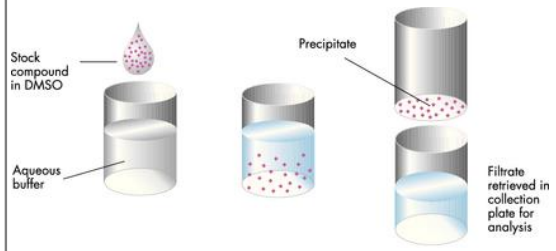


Skin bioavailability is a function of all these factors over time

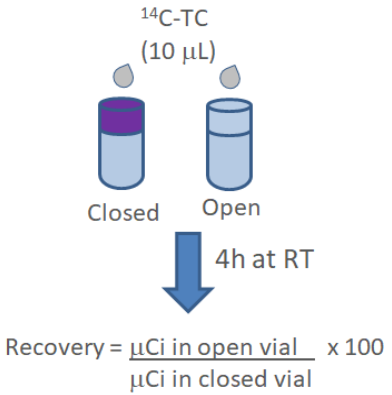
Methods to help address skin bioavailability

Solubility

OECD TG 105



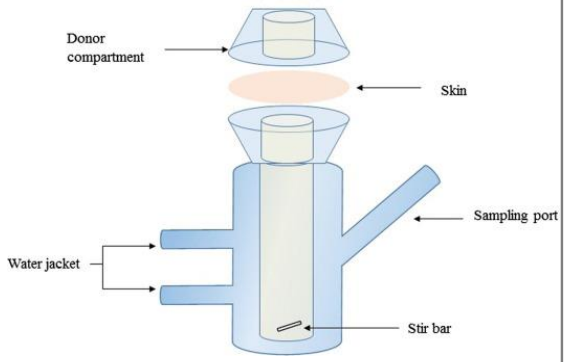
Evaporation



$$\text{Recovery} = \frac{\mu\text{Ci in open vial}}{\mu\text{Ci in closed vial}} \times 100$$

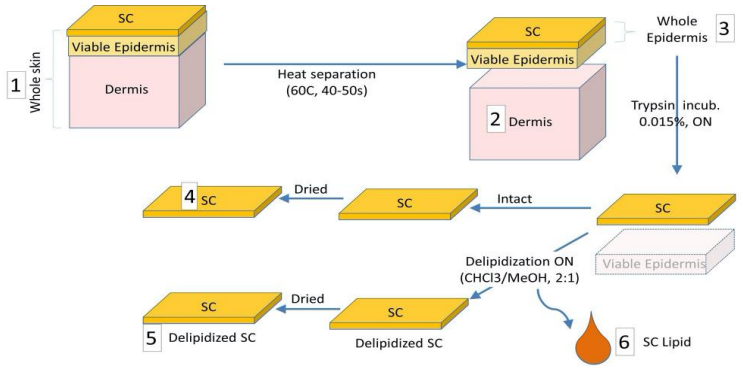
Absorption

OECD TG 428



Metabolism

Distribution



Reactivity

OECD TG 442c

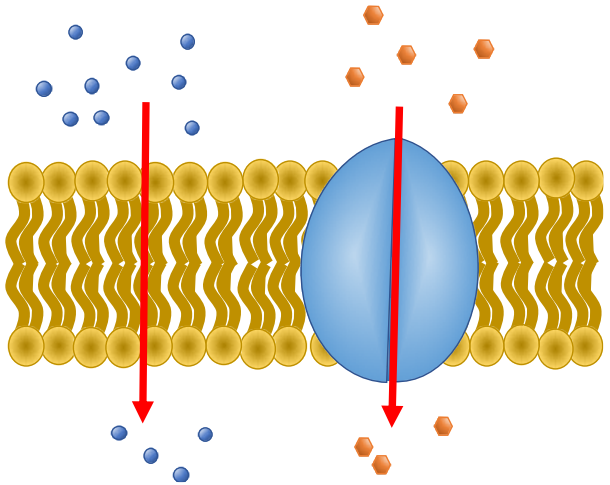
Peptide reactivity assays

Components of Dosimetry: Distribution

Distribution: The uptake of chemicals into the body.

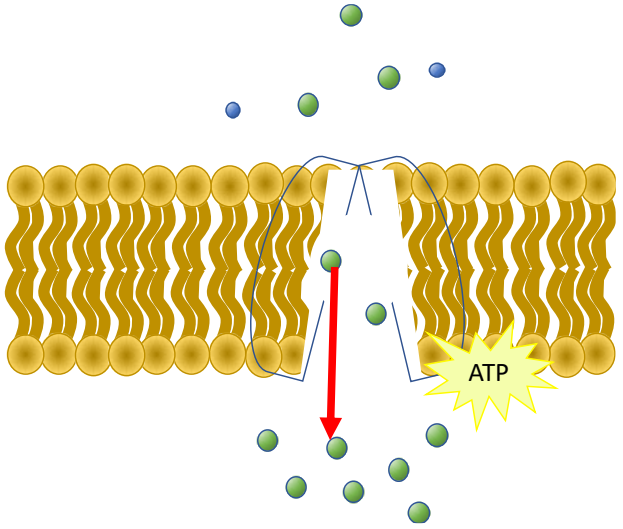
- May be **passive diffusion** or **active transport** between blood & tissues

Passive diffusion



VS

Active transport



Chemical gradient-driven

Requires energy to move chemical against gradient

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Distribution: Determinants of chemical distribution

CHEMICAL DETERMINANTS

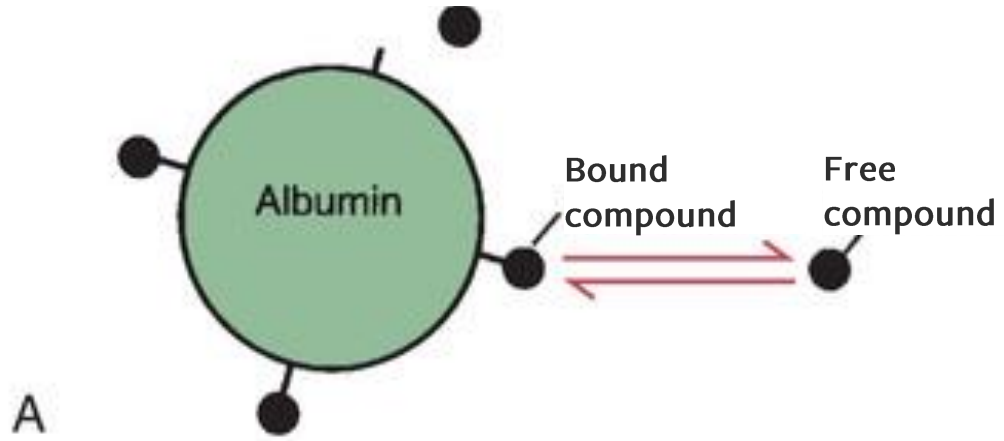
- Log P (lipophilicity) – e.g., chemicals with positive log P are lipophilic
- Fraction of chemical bound to plasma proteins
- Binding to transporters in tissues

PHYSIOLOGICAL DETERMINANTS

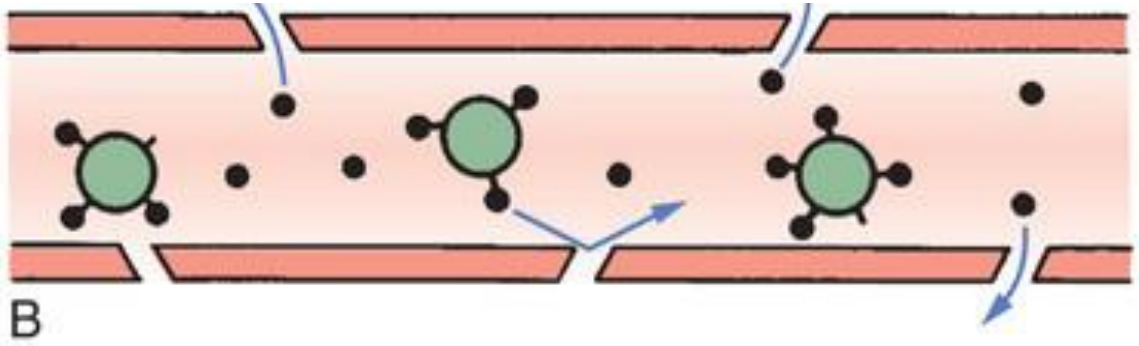
- Body weight
- Tissue volume
- Blood flow to tissues

Distribution: Plasma Protein Binding

Reversible Binding of a Compound to Albumin



Retention of Protein-Bound Compound within the Vasculature



Fraction unbound:

fraction total blood chemical NOT bound to serum proteins

$$fu = \frac{[Free]}{[Total]}$$

[Total] = concentration of total chemical in blood, including both bound and unbound (free) chemical

[Free] = free concentration in blood

fu = fraction unbound, which can range from 0 to 1 (or 0 – 100%)

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Components of Dosimetry: Metabolism

Metabolism: Enzyme mediated transformation of the chemical, in general metabolism alters its physicochemical properties to promote excretion from the body

- Sites of chemical metabolism:
  - → Primarily in the liver, but also in kidney, skin, lung, brown fat and other tissues where specific enzymes are expressed support tissue health and function.
- Effect on chemical toxicity:
  - → Generally promotes clearance
  - → Bioactivation
  - → Inactivation

Liver Metabolism: *In vitro* models

Subcellular fraction

- ✓ Easy to use
- ✓ Amenable to HT
- ✓ Low cost
- ✓ Phase I / II enzymes
- ✓ Clearance, inhibition, binding
- ✓ Pool donors

- ▪ Difficult in vivo extrapolation

Monoculture

- ✓ Easy to use
- ✓ Amenable to HT
- ✓ Commonly used
- ✓ Multiple species
- ✓ Pooled hepatocytes

- ▪ Functional decline ~ 4 hr
- ▪ Lack of fidelity to in vivo structure
- ▪ Difficult in vivo extrapolation

3-D cell culture

- ✓ In vivo-like physiology
- ✓ In vivo-like expression of enzymes
- ✓ Concurrent toxicity evaluation
- ✓ Long term culture

- ▪ Low – medium throughput
- ▪ Can be difficult to image
- ▪ Expensive

Liver slices

- ✓ In vivo-like architecture
- ✓ In vivo-like enzyme & transporter expression

- ▪ Functional decline <24 hr
- ▪ Complicated to use
- ▪ Tissue availability
- ▪ Low throughput
- ▪ Expensive

Predictability, complexity, cost

Ease of handling, reproducibility, throughput

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Components of Dosimetry: Elimination (aka Excretion)

Excretion: Elimination of the chemical from the body

Major Excretion Pathways:

- Renal excretion (kidney urine)
- Fecal excretion (GI, feces, includes contribution from liver via bile),
- Lung (blood, exhaled air)

Other Excretion Pathways

- Hair
- Menstruation
- Lactation
- Sweat, etc.

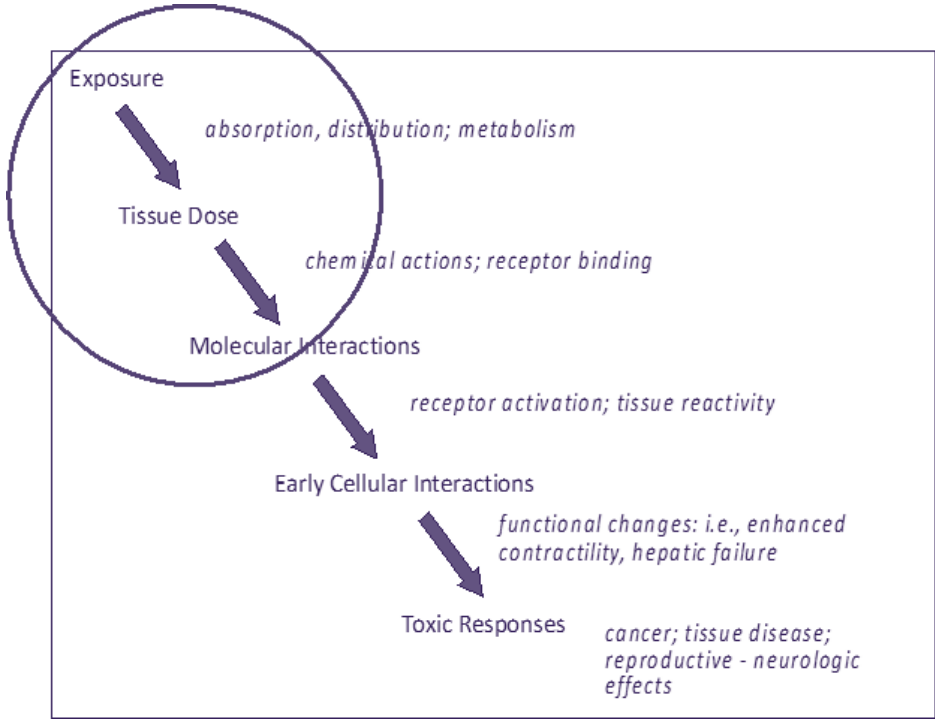
Elimination: Urinary excretion – incorporation in models

- Irreversible transfer of a chemical or its metabolites from plasma into urine
- No *in vitro* methods available
- Active and passive transport can lead to reabsorption
- If renal excretion is predicted to be the dominant route of excretion, it can be calculated as follows:

Renal clearance = Glomerular filtration rate X Fraction unbound X Concentration

Recap: Biokinetics

- Biokinetics is the study of the metabolism and transport of drugs (pharmacokinetics) or chemicals (toxicokinetics) through the body.
- Despite protective barriers such as skin, there are multiple routes of entry for chemicals, including the GI tract and the lungs.
- Once the chemical enters the body, the processes of absorption, distribution, metabolism, and elimination dictate the distribution of a chemical. Measuring these processes helps determine the internal dose of a chemical.



The internal concentration at the organ, tissue, cells and macromolecular levels can have toxic effects. PBK modelling can be used to describe the quantitative relationship between external dose and internal dose.

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Biokinetic Modeling

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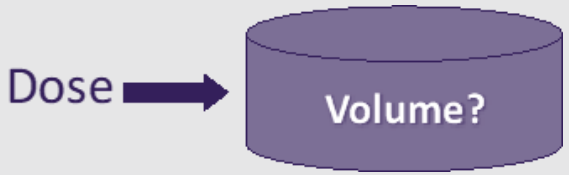
Compartmental models

- Compartmental modelling attempts to describe organs or systems of the body as simple volumetric compartments.
- Compartmental models use the principles described in the previous slides to define a **volume of distribution** for a drug. (i.e., how extensively the drug is distributed in the body).

Example of a 1 Compartment Volume of Distribution Model



- A = amount dosed (ng)
- C = concentration of chemical in the compartment (e.g., blood) (ng/mL)
- V = volume of compartment (ng/mL)
- Assume: bolus dose, i.e., instantly available

Since C = A/V, then V = A/C

C = ~~ng~~/(~~ng~~/mL) -> mL

Body is ~ 75% water

- **Large V:** chemical is distributed widely
- Likely distributed with body water

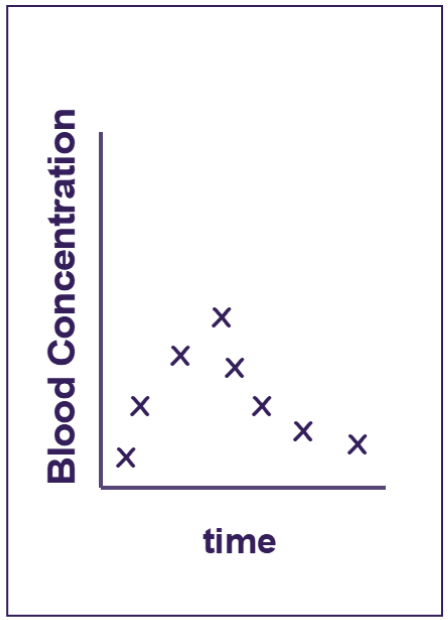
- **Small V:** chemical is poorly distributed
- Likely bound to proteins in the blood (if absorbed)

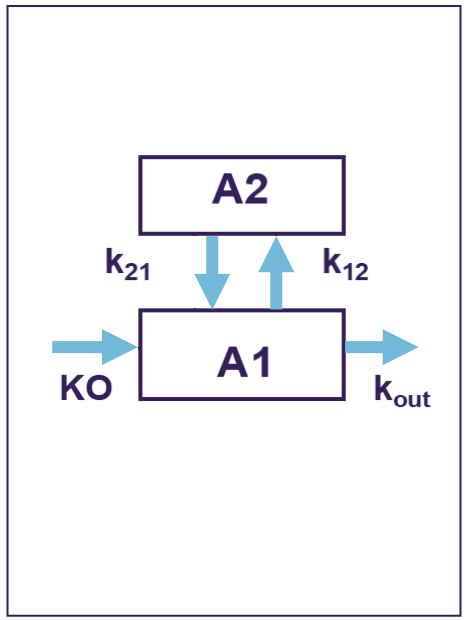
However, this example doesn't account for:
(1) absorption
(2) transport into tissues
(3) clearance

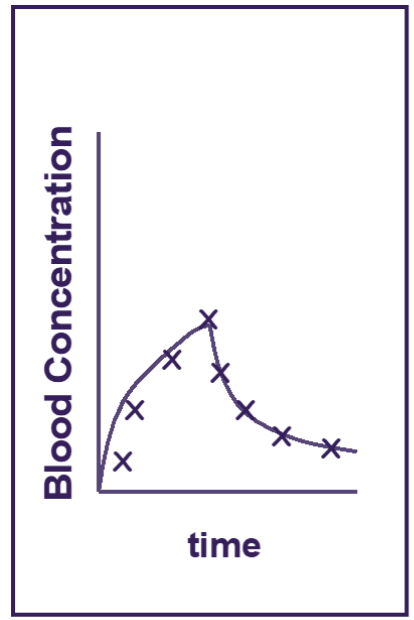
More complex compartmental models combine these processes to describe chemical kinetics in the blood.

Building a classical compartmental PK model

- The rate equations described here can be combined to describe chemical movement in the body
- This can be used to develop an empirical description of drug kinetics, i.e., pharmacokinetics







A1 = amount of drug in compartment 1

A2 = amount of drug in compartment 2

K0 = zero order rate of dosing

Kout = first order rate of elimination (i.e., urine)

K12 = first order rate from compartment 1 to compartment 2 (eg, blood -> tissues)

K21 = first order rate from compartment 2 to compartment 1 (e.g., tissues -> blood)

Rates are fitted to measured time course data.

Benefits and limitations to compartmental models

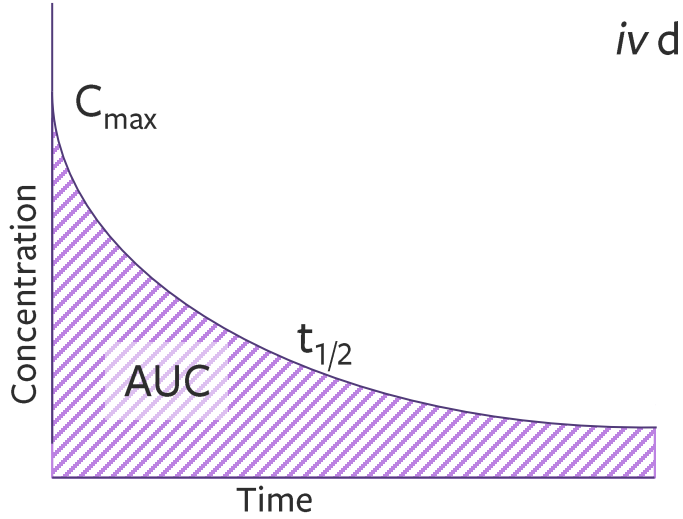
BENEFITS

- Simple to create
- Many existing software options
- Allows calculation of informative parameters
  - AUC = area under the curve = average concentration
  - Cmax = max concentration
  - Tmax = time at which drug reaches Cmax
  - t/2 = half-life
  - calculate time to steady state (esp. for slowly cleared chemicals)

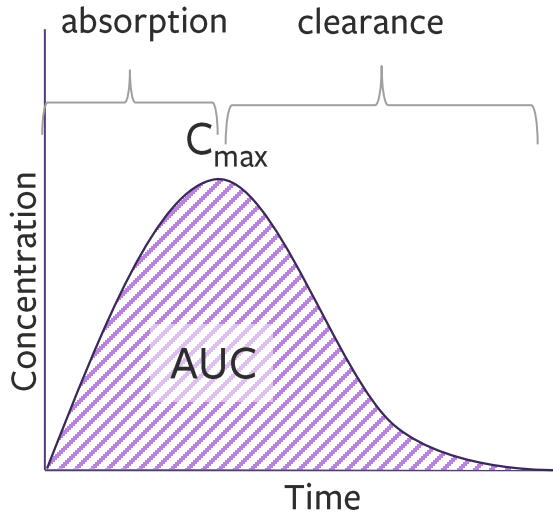
LIMITATIONS

- Cannot extrapolate outside experimental conditions
- E.g., different animals, doses, dose routes, etc.
- Can only describe data, does not predict behavior

iv dose



Non-iv dose (*po*, *sc*, etc)



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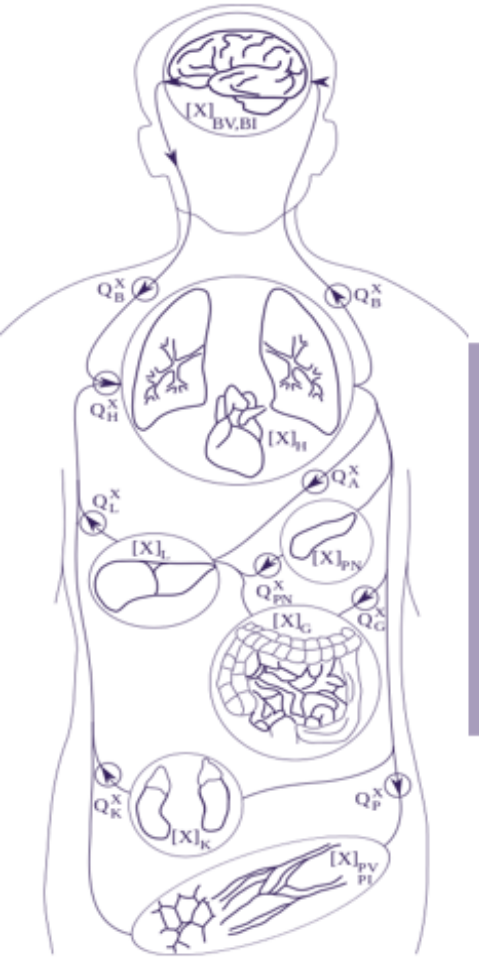
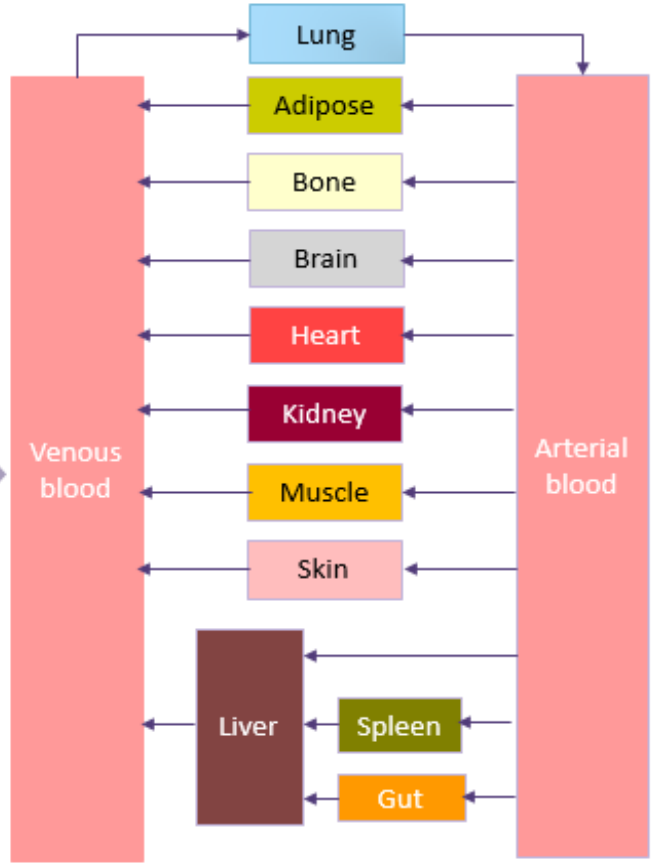
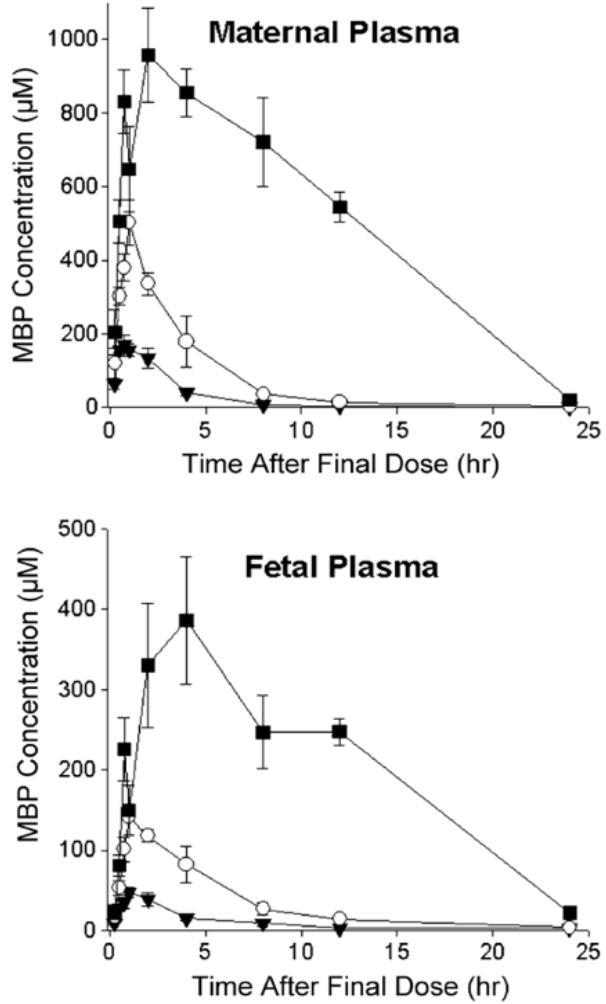
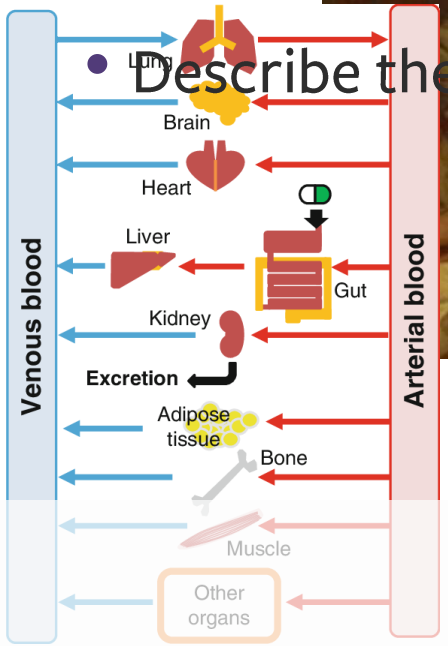
PBK models

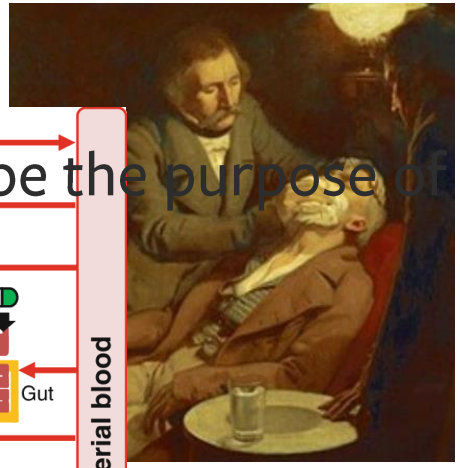



Lung
Adipose
Bone
Brain
Heart
Kidney
Muscle
Skin
Liver
Spleen
Gut
Venous blood
Arterial blood


Maternal Plasma
MBP Concentration (µM)
Time After Final Dose (hr)
Fetal Plasma
MBP Concentration (µM)
Time After Final Dose (hr)

Many names for a common process

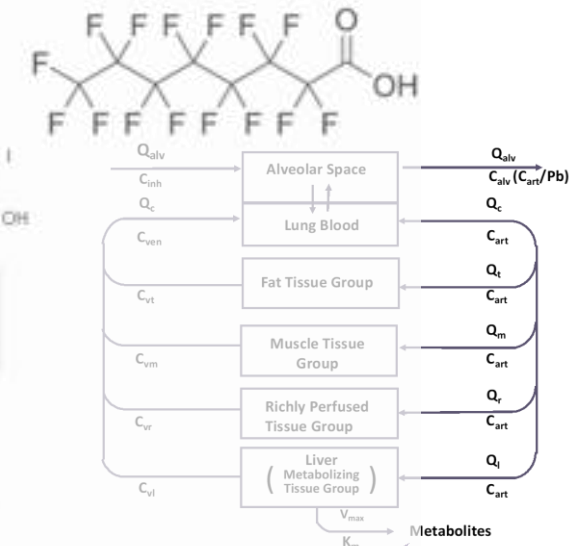




- Describe the purpose of PBK

PBPK Model

(Physiologically-Based Pharmacokinetic)



PBK model course content overview

- Why use PBK models?
- Building a PBK model
  - → Defining structure
  - → Parameterization
- Validation
  - → Comparison to data
  - → Sensitivity and uncertainty analysis
- Applications of PBK
  - → Extrapolation
    - Species, route of exposure, life stage, acute -> chronic exposures
  - → Target tissue dosimetry
  - → Interpretation of Biomonitoring
  - → *In vitro* to *in vivo* extrapolation (IVIVE)

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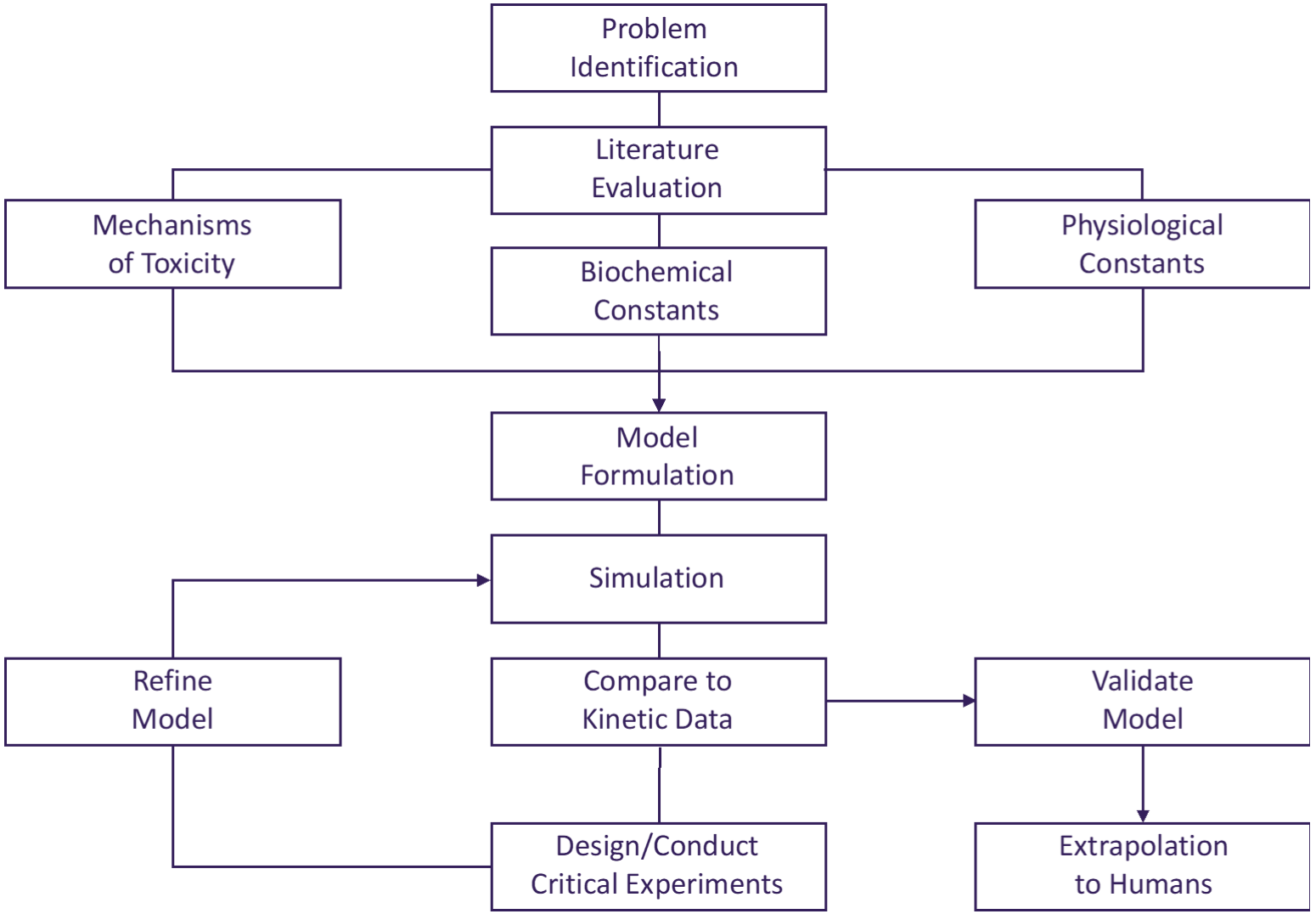
Why PBK models?

- Extrapolation (predicting beyond the experimental conditions) rather than interpolation (describing kinetics within the experimental conditions) as in classical PK
  - → Route
  - → Species
  - → Life stage, gender, etc.
  - → Single to repeated dose
  - → Chemical
- Pharmacokinetic modelling is a valuable tool for evaluating tissue dose under various exposure conditions in different animal species.
- To develop a full picture of the biological responses caused by exposure to toxic chemicals, it is necessary to analyze the processes that will determine your tissue dose and the interactions of chemical with tissues.
- Physiological modelling approaches are used to uncover the biological determinants of chemical disposition

PBK modelling process: Iterative development



Clewell, R. A., and Clewell H. J. (2008). Regul. Toxicol. Pharmacol. 50(1), 129-143. PMID: 18077066.

Occam's Razor

"Entities should not be multiplied without necessity"

OR

'The simplest explanation is usually the right one'

Modelling is inherently a balance between including necessary detail and keeping the model tractable.

Ask yourself:

1. Is this biologically plausible?
2. Is it necessary to describe the chemical kinetics?
3. Is there sufficient data to parameterize it?
4. Can I test or validate the parameters/description?

"All models are wrong, and some are useful."

- George Box

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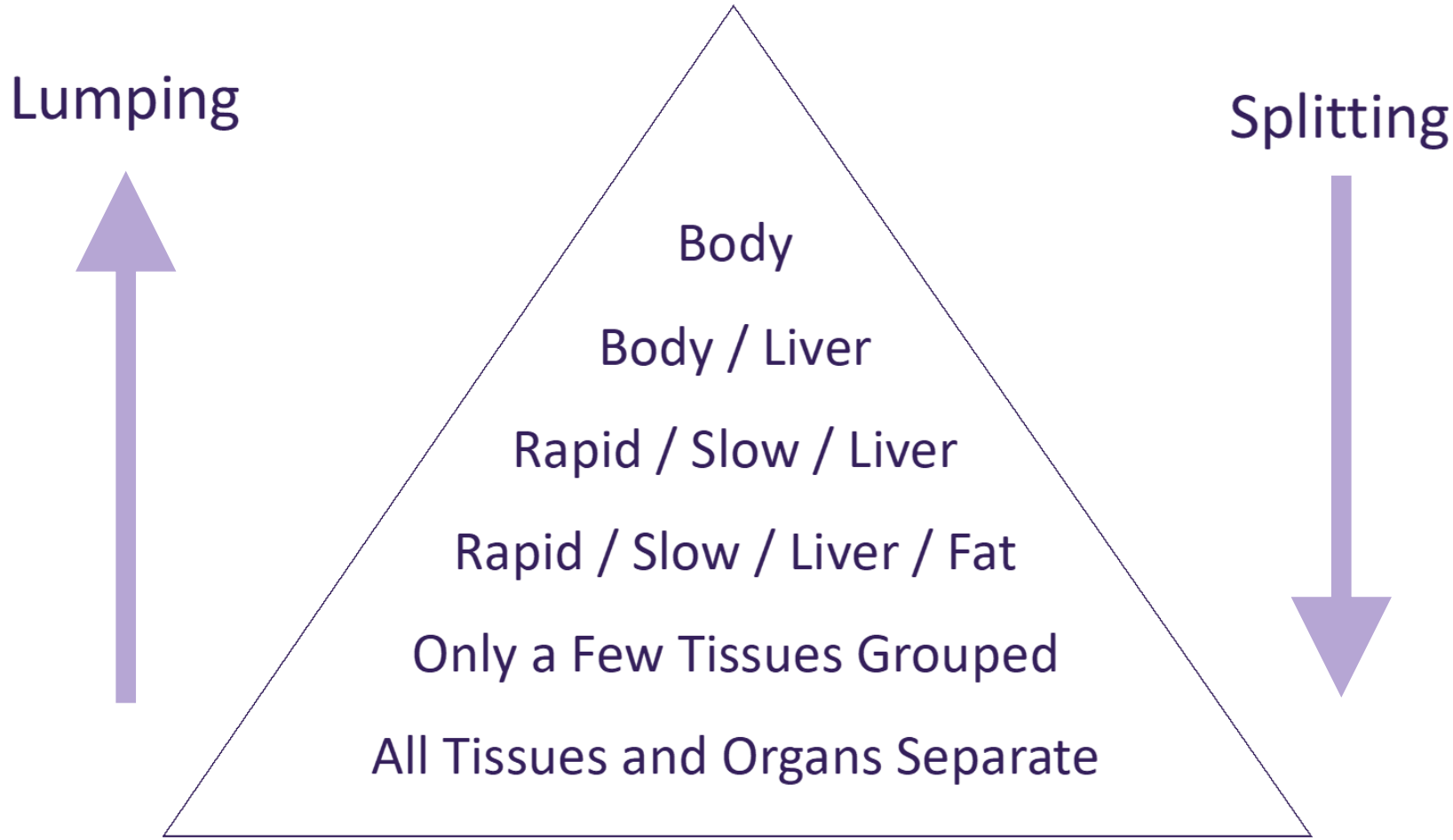
Modeling philosophy: Lumping vs. splitting



PBK model: Tissue grouping

Tissue Grouping Criteria

- perfusion rate = blood flow / volume
  - time constant (R): units = hr^{-1}
- R = Q / V
- “rapidly” perfused tissues
  - E.g., gut, liver, kidney, etc.
- “slowly” perfused tissues
  - E.g., muscle, skin, fat

Model purpose

- Target tissue
- Description of a particular process
  - Metabolism
  - Enterohepatic recirculation
  - Protein binding
  - Active transport, etc.
- Important for ADME

Should be fit for purpose!

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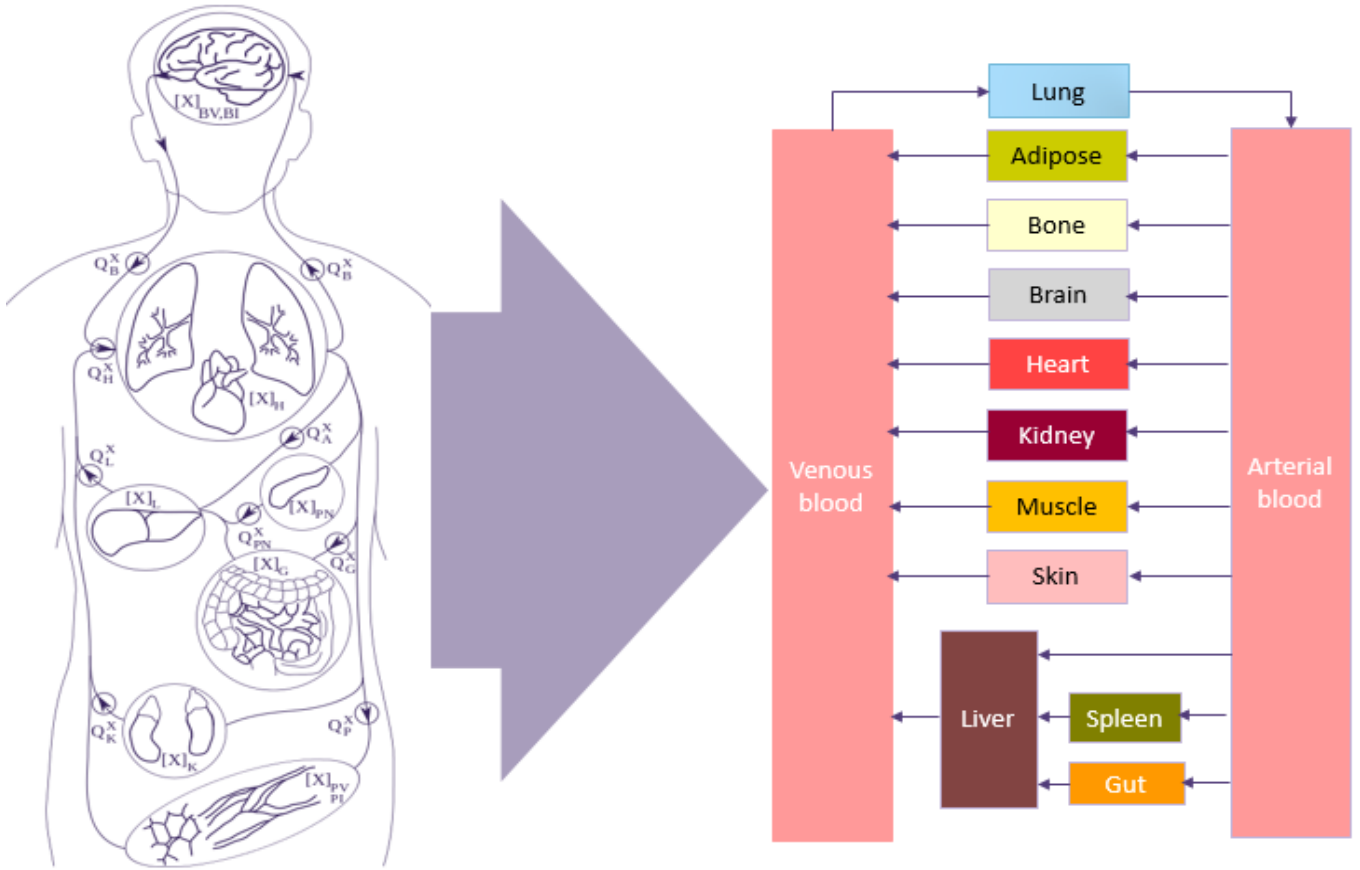
PBK model: Compartments

- Storage
  - → Fat, RBCs,...
- Excretion
  - → Urine, feces, milk, hair, ...
- Flow-limited metabolism
  - → Liver, kidney, skin,...
- Uptake routes (e.g., skin)
  - → Skin, GI, blood, lungs....
- Target Tissues
  - → Any
- Distributional kinetics
  - → Any



PBK model: Deciding which tissues to include

- Target Tissues
  - ✓ metabolism
  - ✓ binding
  - ✓ Pharmacodynamics
- Metabolite Compartments
  - ✓ compartmental description
  - ✓ physiologically based description
- Experimental Apparatus
  - ✓ chamber
  - ✓ sampling device
- Experimental problems
  - ✓ Loss of material
  - ✓ Preening
- Total radioactivity data
  - ✓ Represents sum of parent and metabolite concentrations
  - ✓ May require “other metabolites” compartment
- Tracer data
  - ✓ If kinetics are dose-dependent, need to model both unlabeled and labeled material
  - ✓ Similar problem for endogenous compounds
- Multiple chemical interactions
  - ✓ Competition
  - ✓ Inhibition/induction

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PBK model: Description of a single tissue

Mass balance equation: $\frac{dA_T}{dt} = A' = Q_T * C_A - Q_T * C_{VT}$

$$A_T = \int A' \; dt$$

$$C_T = A_T / V_T$$

$$C_T = C_{VT} / P_T$$

Q_T = tissue blood flow

C_A = arterial blood concentration

C_V = venous blood concentration

P_T = tissue partition coefficient

V_T = tissue volume

A_T = amount of chemical in tissue

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PBK model: Parameters

PHYSIOLOGICAL PARAMETERS

- Body weight
- Cardiac output
- Organ weight or volume
- Blood flow rate
- Vascular space of each organ
- Tissue composition

Data for these can be found in references given in the resources section

CHEMICAL-SPECIFIC PARAMETERS

<u>Physicochemical</u>
- Molecular formula
- CAS no.
- Molecular weight
- LogP
- pKa
- Solubility

<u>Absorption</u>
- Skin absorption rate
- Evaporation rate/ vapor pressure

<u>Distribution</u>
- Fraction unbound in plasma (FuP)
- Ratio Blood to Plasma (Rbp)
- Volume of distribution

<u>Metabolism and Excretion</u>
- Hepatic Clearance rate (CL)
- Vmax and Km

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PBK Model: Validation

Validation includes:

- evaluation of the fit to data,
- predictive capability for validation data (data not used for model building)
- appropriateness of model structure
- appropriateness of model parameters
- parameter sensitivity vs. parameter uncertainty

Applicability Domain	Uncertainty	Variability	Validation	Reporting

For more: see AFSA E&T Module 3 and OECD (2021), Guidance document on the characterisation, validation and reporting of Physiologically Based Kinetic (PBK) models for regulatory purposes, OECD Series on Testing and Assessment, No. 331, Environment, Health and Safety, Environment Directorate, OECD.

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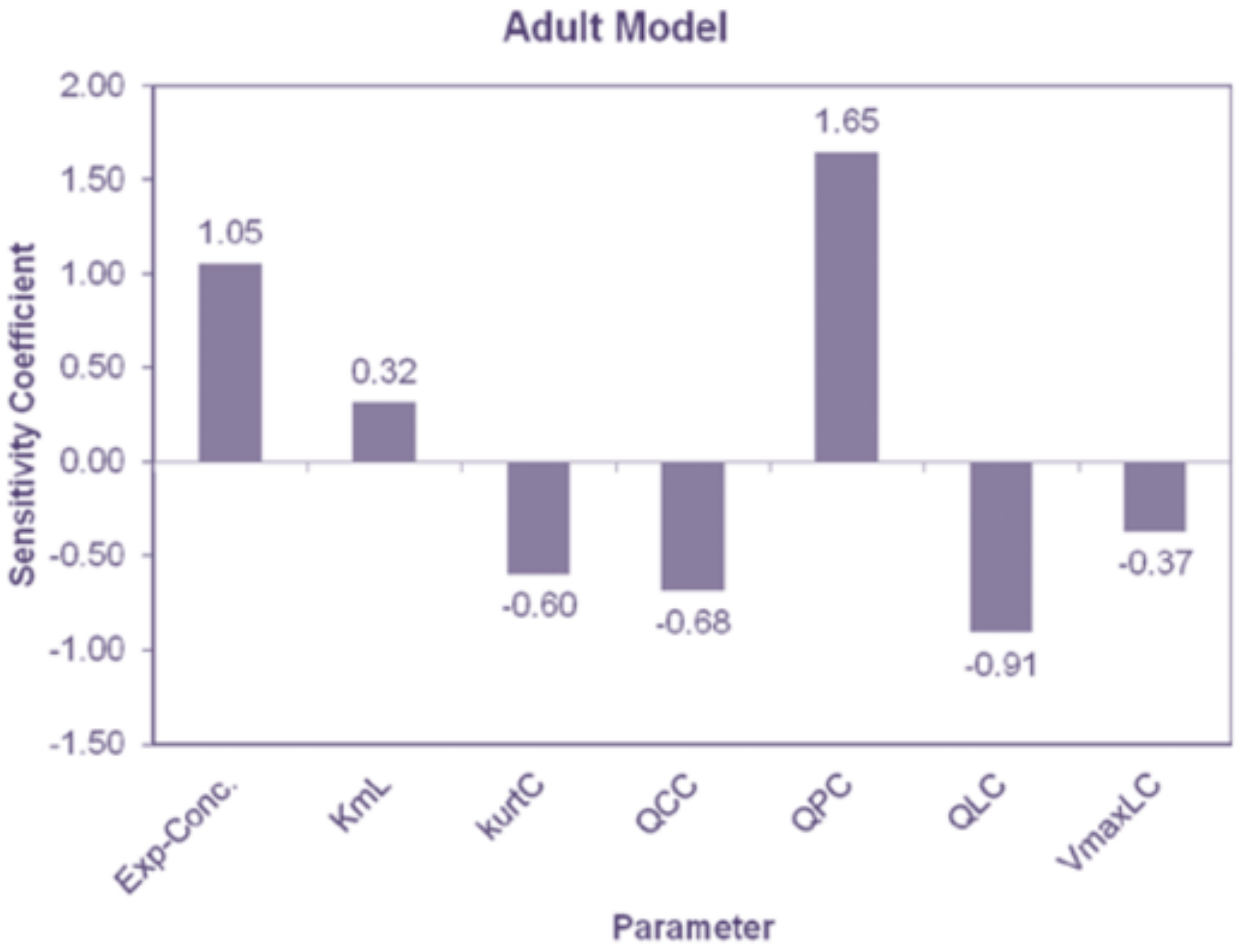
PBK model validation: Sensitivity analysis

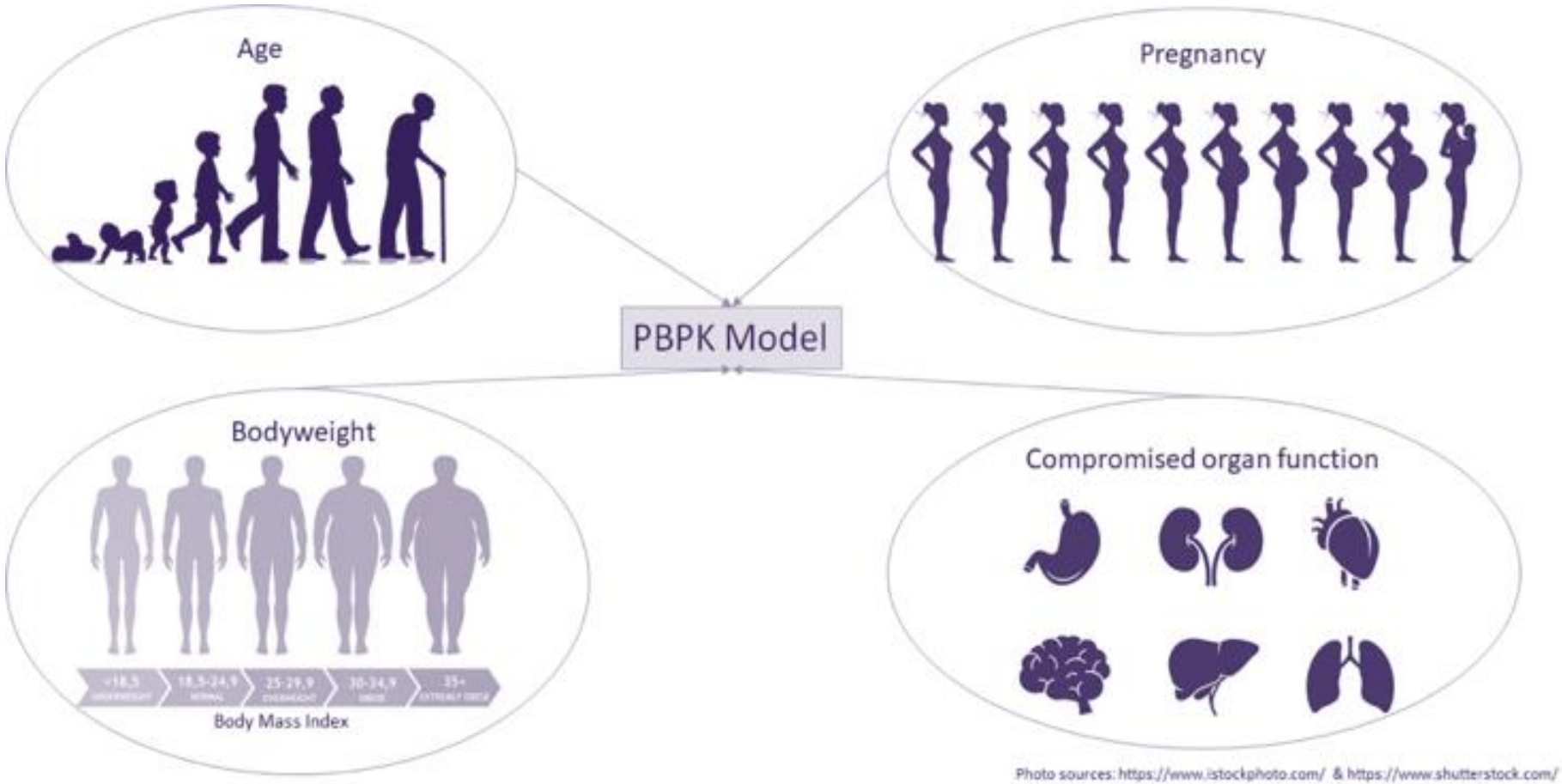
Sensitivity analysis for PBK model for ethanol in the adult rat



1% change in parameter vs. change in blood ethanol concentration

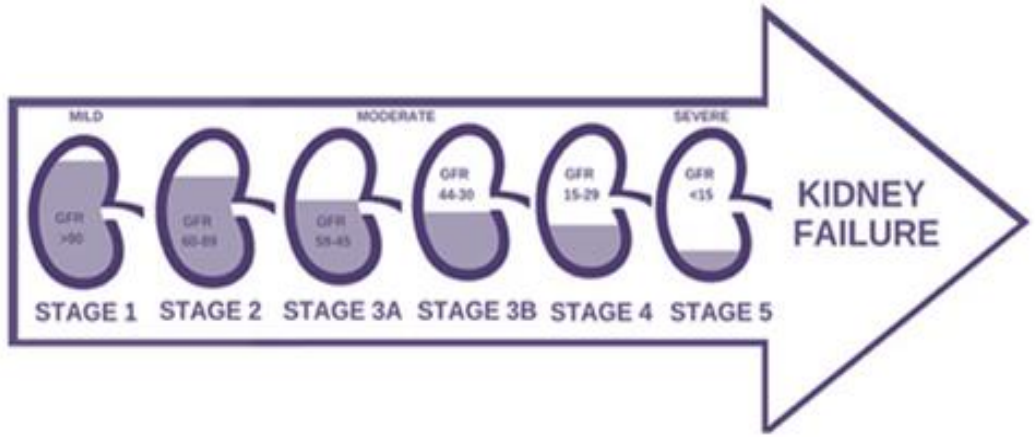
Martin SA et al. 2012. Inhal Toxicol. 24(11):698-722.

PBK model application: Life-stage extrapolations



PBK Model Applications: Account for Disease States

- Example: Renal Impairment



Step 1: Develop and qualify PBK model for each drug in health subjects

↓

Step 2: Predict PK for each drug in various renal impairment populations

↓

Step 3: Calculate AUC ration (AUCR) between renal impairment and healthy subjects based on PBK and static models

↓

Step 4: Compare model (PBK/static)-predicted AUCR with observed values in renal impairment studies across all drugs

Yee KL et al. (2019) https://www.dneph.com/chronic-kidney-disease/stages-of-ckd/

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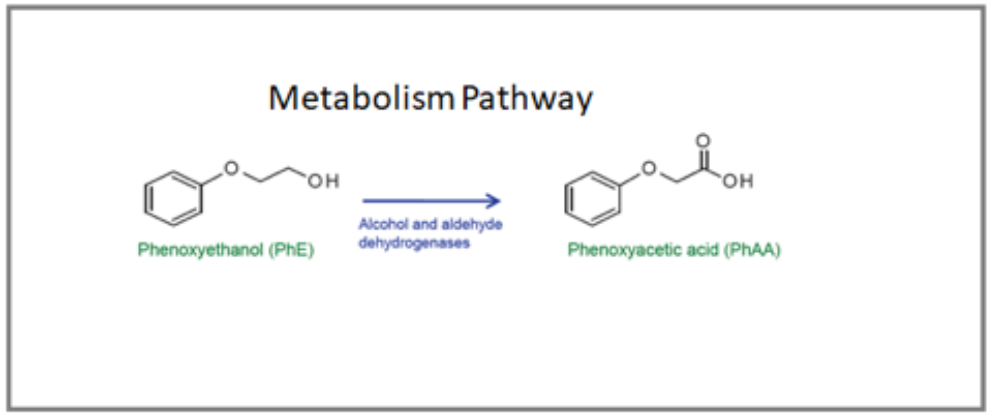
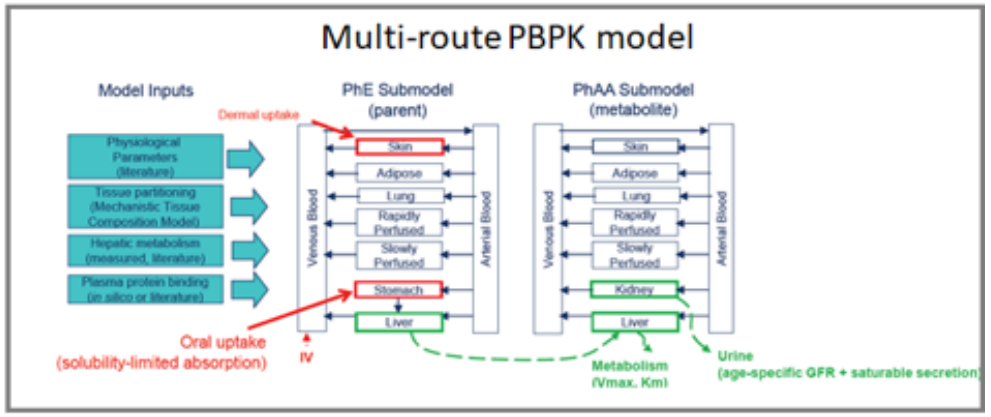
PBK Model Applications: Aggregate exposure

- Example - Phenoxyethanol



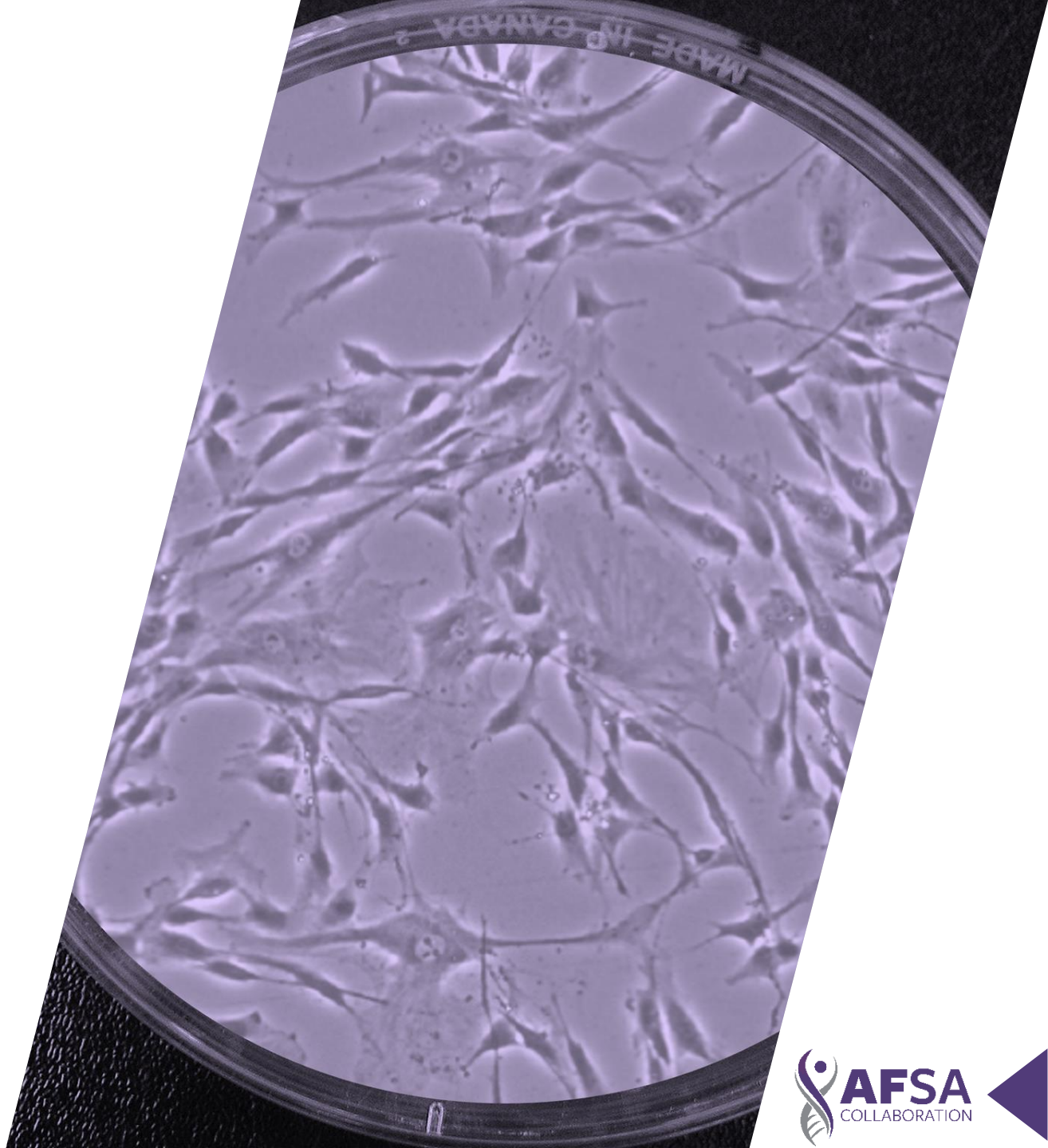
Product	Product use (g/day)	Retention Factor	Daily dose (mg/kg/day)
Shower gel	0.19	0.01	0.02
Hair conditioner	0.11	0.01	0.01
Shampoo	0.04	0.01	0.01
Hair styling	0.40		0.05
Liquid foundation	0.51	1.0	0.06
Makeup remover	0.50	0.1	0.07
Hand wash - soap	0.20	0.01	0.03
Body lotion	7.82	1.0	0.99
Face cream	1.54	1.0	0.19
Hand cream	2.16	1.0	0.26
Deodorant non-spray	1.50	1.0	0.18
Eye makeup	0.02	1.0	0.00
Mascara	0.03	1.0	0.00
Lipstick	0.06	1.0	0.01
Eyeliner	0.01	1.0	0.00
Toothpaste	0.14	0.05	0.02
Mouthwash	2.16	0.1	0.29
Total	17.38		2.19



Aggregate Internal Exposure

Subpopulation	Description	External dose	BW (kg)	Internal dose metric			
				AUC (mg*h/L or mg-Equiv*h/L)		Cmax (mg/L or mg-Equiv/L)	
		mg/kg/day		PhE	PhAA	PhE	PhAA
Adult Human	Aggregate (oral + cosmetics)	2.69	60	0.608	8.82	0.549	1.11

IVIVE



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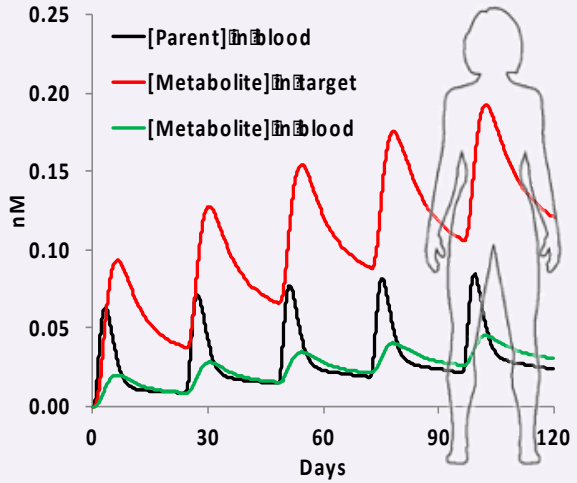
Traditional vs. NAM-based toxicology

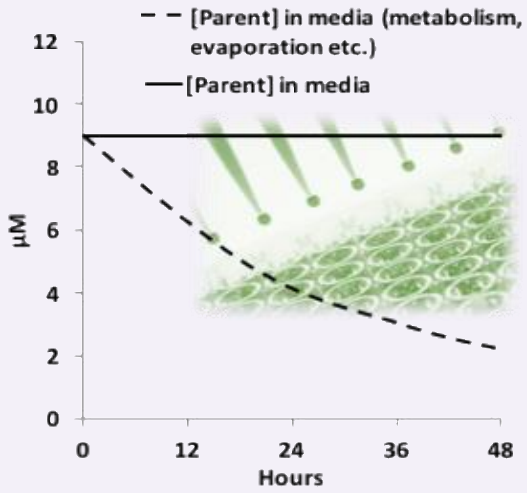
TRADITIONAL TOXICOLOGY

- Test in animals --> predict in humans
- Translate animal Dose-Response to human exposure
- PBPK models predict human dosimetry from animal data and comparative physiology/biochemistry



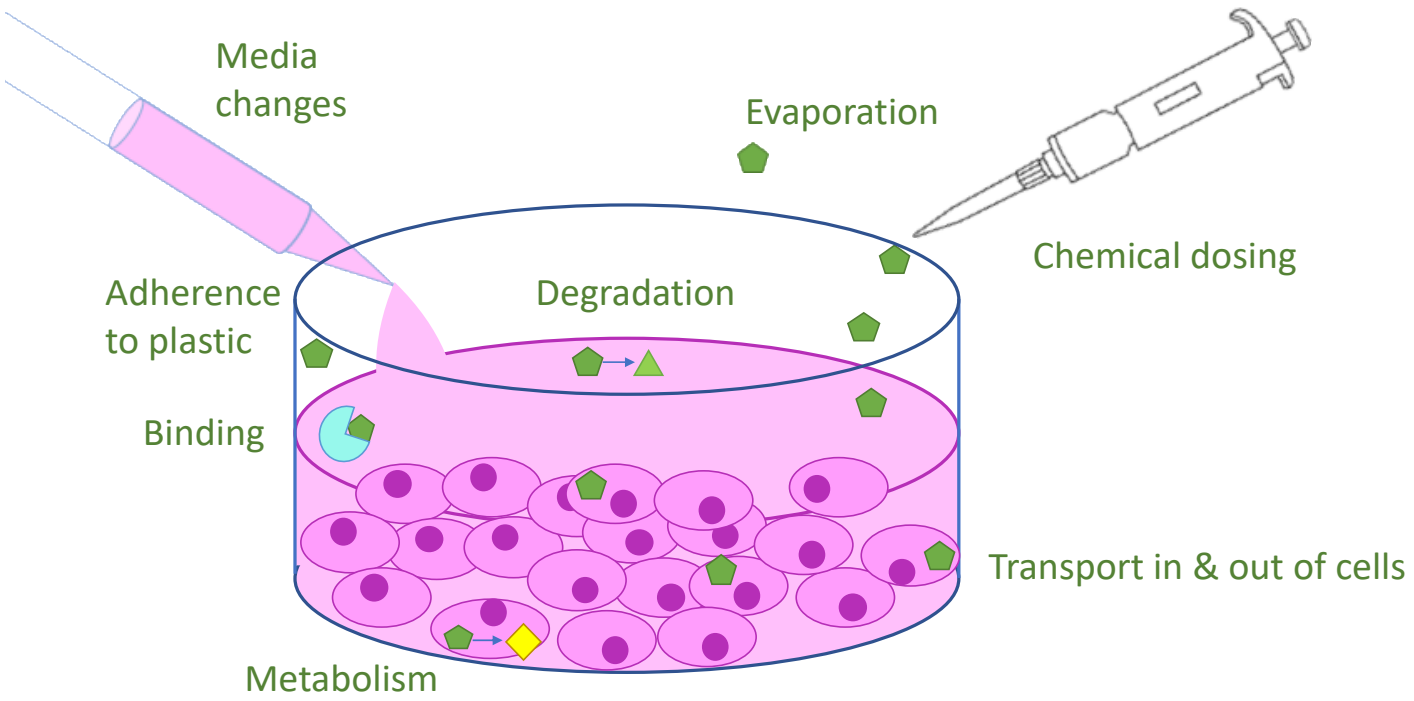
21ST CENTURY TOXICOLOGY

- Test human in vitro --> predict in vivo
- Translate dose in cell culture medium to human exposure
- Modified PBPK models (aka IV-IVE) predict human dosimetry from physicochemical properties, in vitro kinetics and human physiology

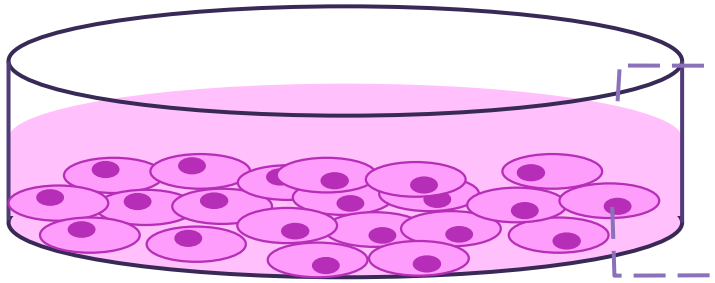


In vitro kinetics: Overview

- Cell culture are dynamic systems with many kinetic processes
- The sum of these process determines free chemical concentration and can change over time
- Be aware that actual dose (media concentration) may not equal nominal (expected) concentration!
- Best practice would be to measure media concentration; in reality, rarely done

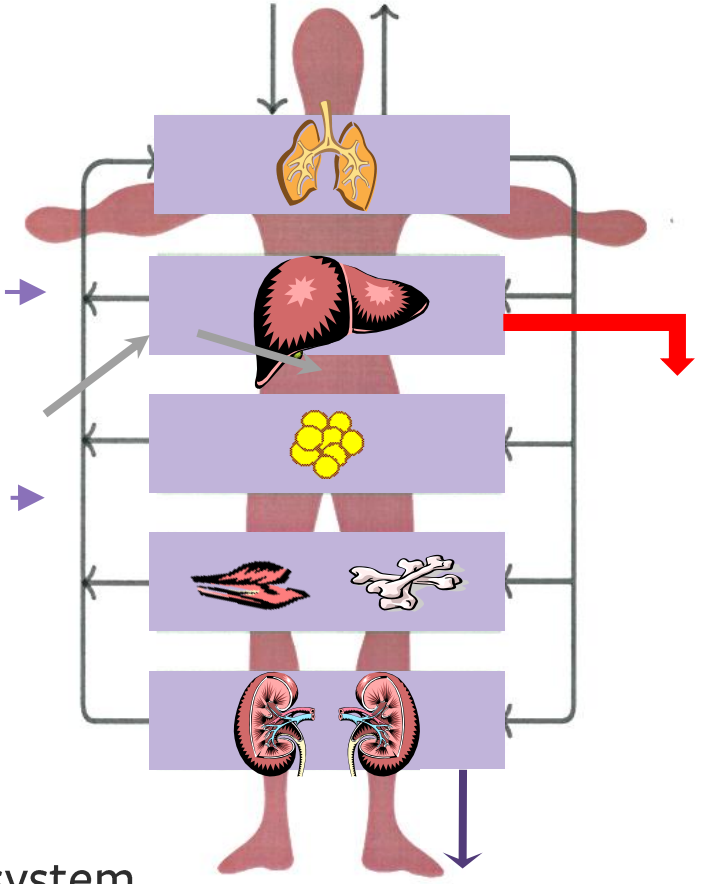


IVIVE: An extension of PBK



Culture medium delivers chemical, like blood

Cultured cells respond to chemical, like tissues



However, some key determinants of dose are missing in vitro:

- Metabolism (e.g. liver)
- Clearance (e.g. kidney)
- Plasma proteins (e.g. albumin)

Two approaches:

1. Engineering- Build system with higher fidelity
2. Computational models – predict system mathematically

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HT-IVIVE: A simplified model for rapid prediction of oral equivalent dose

- OED = estimated oral dose required to cause equivalent effect in humans in vivo as observed in vitro
  - → Aka, administered equivalent dose (AED)

- Considers only the most pertinent kinetic processes affecting steady-state:
  - Absorption
    - → Assume 100%, measure with Caco-2 cells
  - Metabolism
    - → QSAR models, in vitro metabolism assays
  - Urinary clearance
    - → Glomerular filtration
  - Serum binding
    - → In vitro assays

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HT-IVIVE: A simplified model for rapid prediction of oral equivalent dose

$$[\text{Chemical}]_{\text{steady state}} = \frac{\text{Dose rate} * \text{Body weight}}{(Cl_{renal} + Cl_{hepatic})}$$

$$Cl_{renal} = fu * GFR$$

$$Cl_{hepatic} = Q_L * \frac{fu * Cl_{int}}{Q_L + fu * Cl_{int}}$$

$$Cl_{int} = HPGL + V_L * Cl_{in\ vitro}$$

Further reading: Rotroff DM, et al . 2010. Toxicol Sci. 117(2):348-58. PMID: 20639261

Assumptions:

- 100% oral bioavailability
- Linear kinetics (no saturation)

Definitions:

- Cl_{renal} = renal clearance (L/hr)
- $Cl_{hepatic}$ = hepatic clearance (L/hr)
- Cl_{int} = intrinsic clearance (L/hr)
- GFR = glomerular filtration
- *fu* =fraction unbound in plasma
- Q_L = hepatic blood flow (L/hr)
- HPGL = hepatocytes per gram liver
- V_L = liver volume (g)

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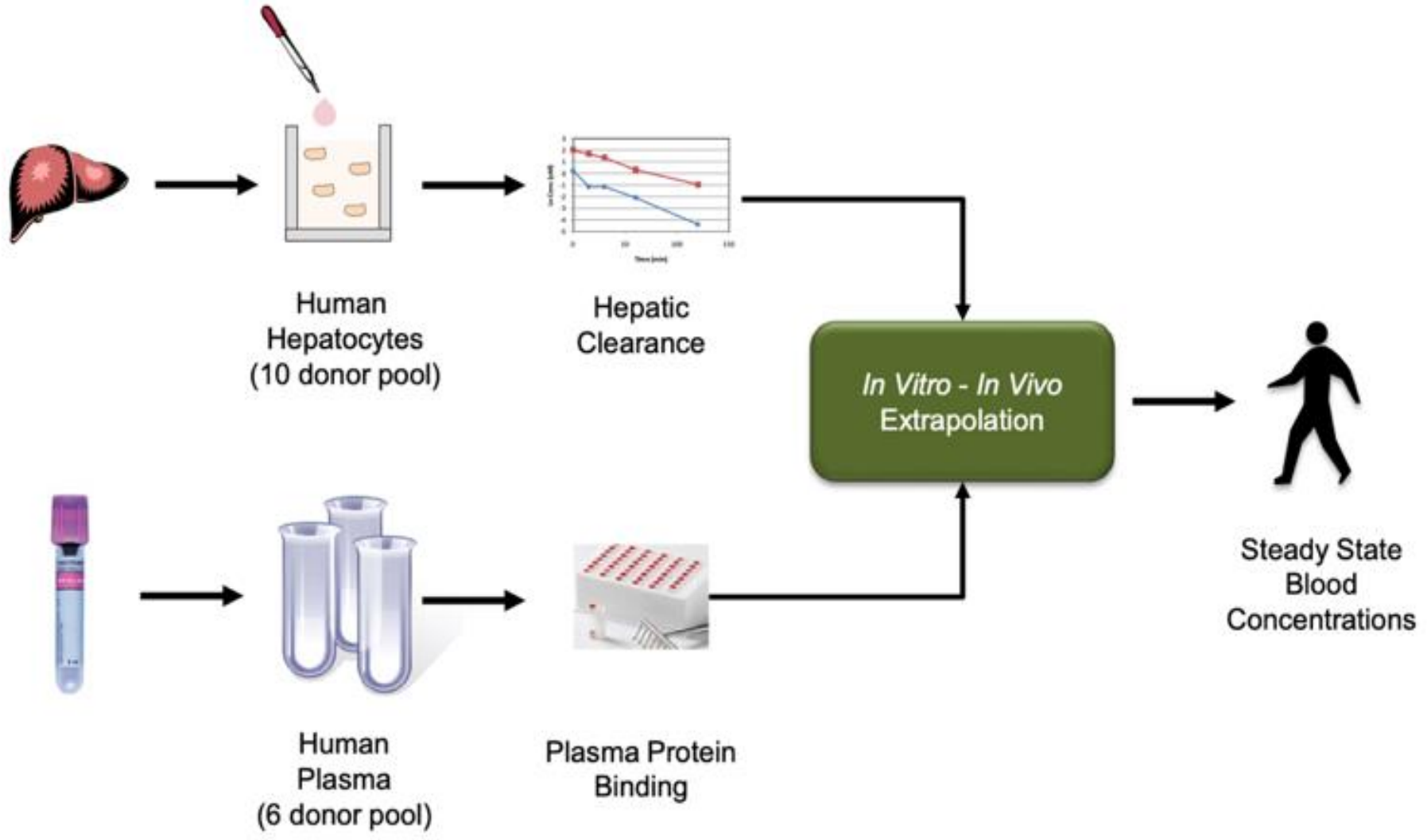
IVIVE: *In vitro* data collection for HT-IVIVE



Rotroff et al., 2010, Toxicol Sci.117(2):348-58. doi: 10.1093/toxsci/kfq220.

IVIVE: Scaling in vitro measurements to *in vivo* values

- Specific activity = $\frac{\textit{enzyme activity}}{\textit{unit enzyme content}}$

enzyme activity - nmol/min

unit enzyme content - mg protein

→ **Specific activity = nmol/min/mg protein**

- Total activity in the system =

specific activity **x** *total enzyme content* in the system

same ↑ different ↑

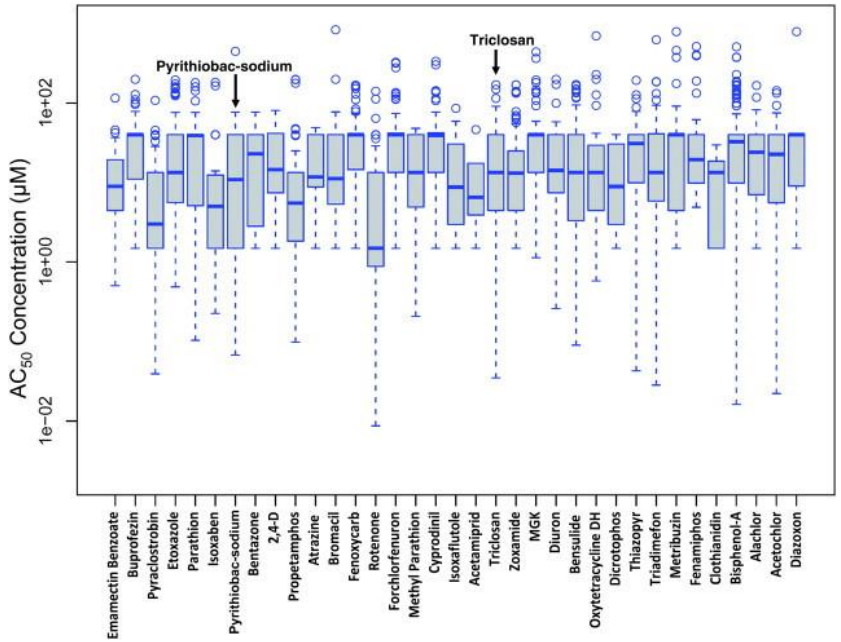
→ SCALING REQUIRED!

IVIVE: Scaling in vitro measurements to in *vivo* values

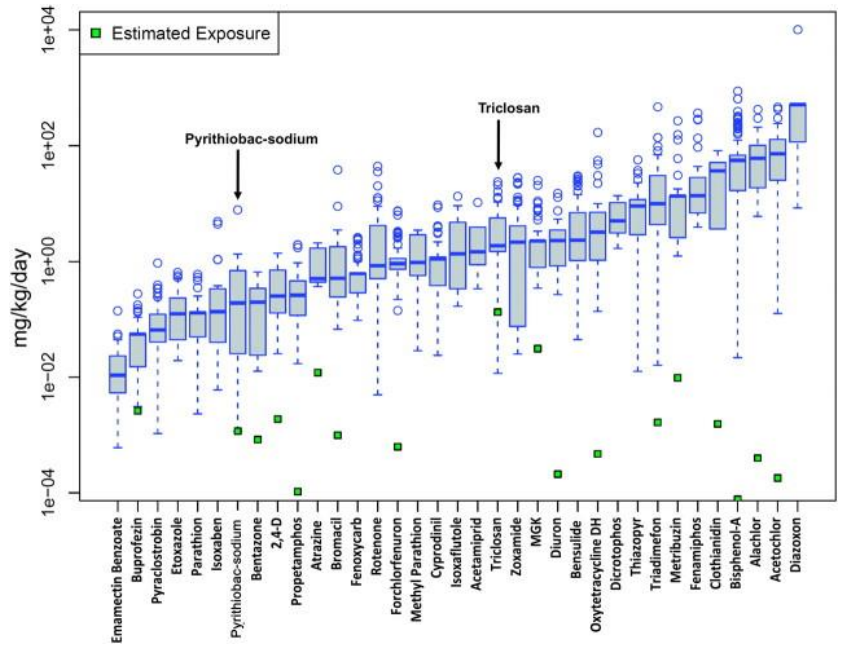
	System	Vmax or Clint in the System	Scaling factors to whole liver
in vitro	**Microsomes**	nmol/min /mg protein	MPPGL x Liver weight
	Cytosol	nmol/min /mg protein	CPPGL x Liver weight
	Expressed Enzyme	µl/min /pmol enzyme	(ISEF x$Enz_{abundance}$) x MPPGL x Liver weight
	Hepatocyte	µl/min /10^6 cells	HPGL x Liver weight
in vivo	**Liver**	nmol/min/g liver	Liver weight
	Whole Body	nmol/min/whole liver	

IVIVE: Application Example
Interpreting high throughput in vitro screening data

- Rapid estimation of margin of exposure (MOE) from in vitro assays *(shown here for ToxCast screening)*



- Identification of chemicals with greater potential for human risk



Wetmore et al., Toxicol Sci. 2010 Oct; 117(2): 348–358.

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IVIVE: Limitations of HT-IVIVE

Assumptions made to simplify the process and allow rapid assessment may not hold true for certain chemicals, such as those with:

- Slow clearance (e.g., coumarin)
- Biding in tissues
- Extrahepatic metabolism
- Bioactivation
- Active transport processes (active uptake into liver/other tissues).
- Complex clearance, transport, etc (e.g., reuptake in kidney, enterohepatic recirculation, lymph distribution
- Fat accumulation (lipophilic)
- Other routes of exposure
- Etc.

See Yoon et al., Toxicology. 2015 Jun 5;332:1-3. doi: 10.1016/j.tox.2015.02.002. Epub 2015 Feb 11.

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IVIVE: Quantitative (Q)-IVIVE

What is it?

More descriptive model that substitutes data for the less precise assumptions used in HT-IVIVE

When do you use it?

- Exceptions to the simple HT-IVIVE
- Poorly metabolized compounds
  - → Systems for long term culture
- Active metabolites
  - → Solutions for metabolite ID
  - → Other approaches to metabolite activity in vitro
- Extra-hepatic metabolism
- Volatile compounds
  - → Inhalation
- Intestinal absorption
- Skin absorption

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IVIVE: In vitro systems to support Q-IVIVE

- Organotypic models to collect parameters
  - → liver bioreactors to collect metabolism parameters for slowly metabolized chemicals)
  - → modified Caco-2 cells with phenotypic transporters
  - → bioactivity assays with metabolic components (S9 fraction, flow through hepatocytes to target cells)
- More complex IVIVE model with additional kinetic processes
  - → dermal absorption
  - → transporter function, etc.

See Yoon et al., Toxicology. 2015 Jun 5;332:1-3. doi: 10.1016/j.tox.2015.02.002. Epub 2015 Feb 11.

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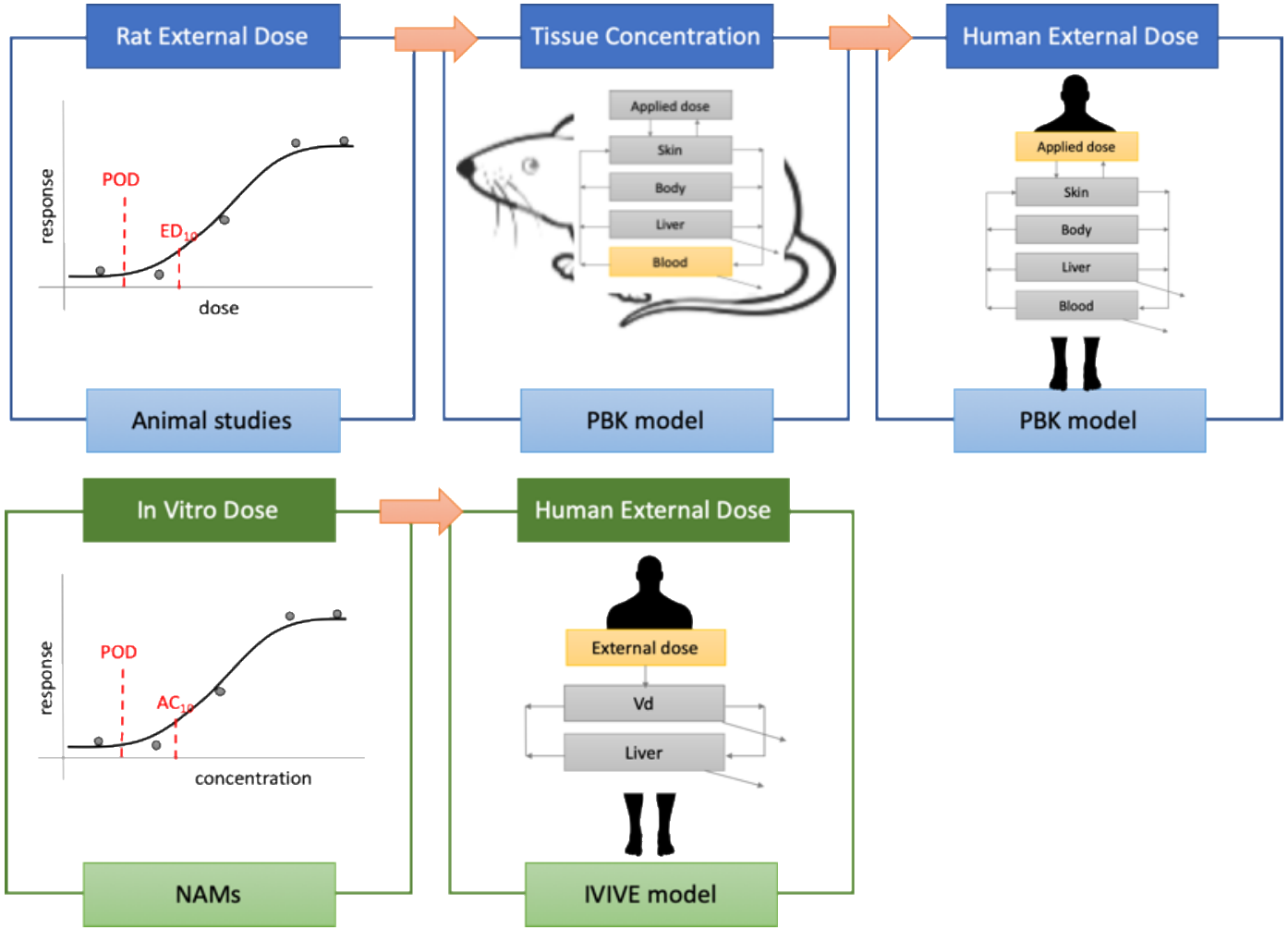
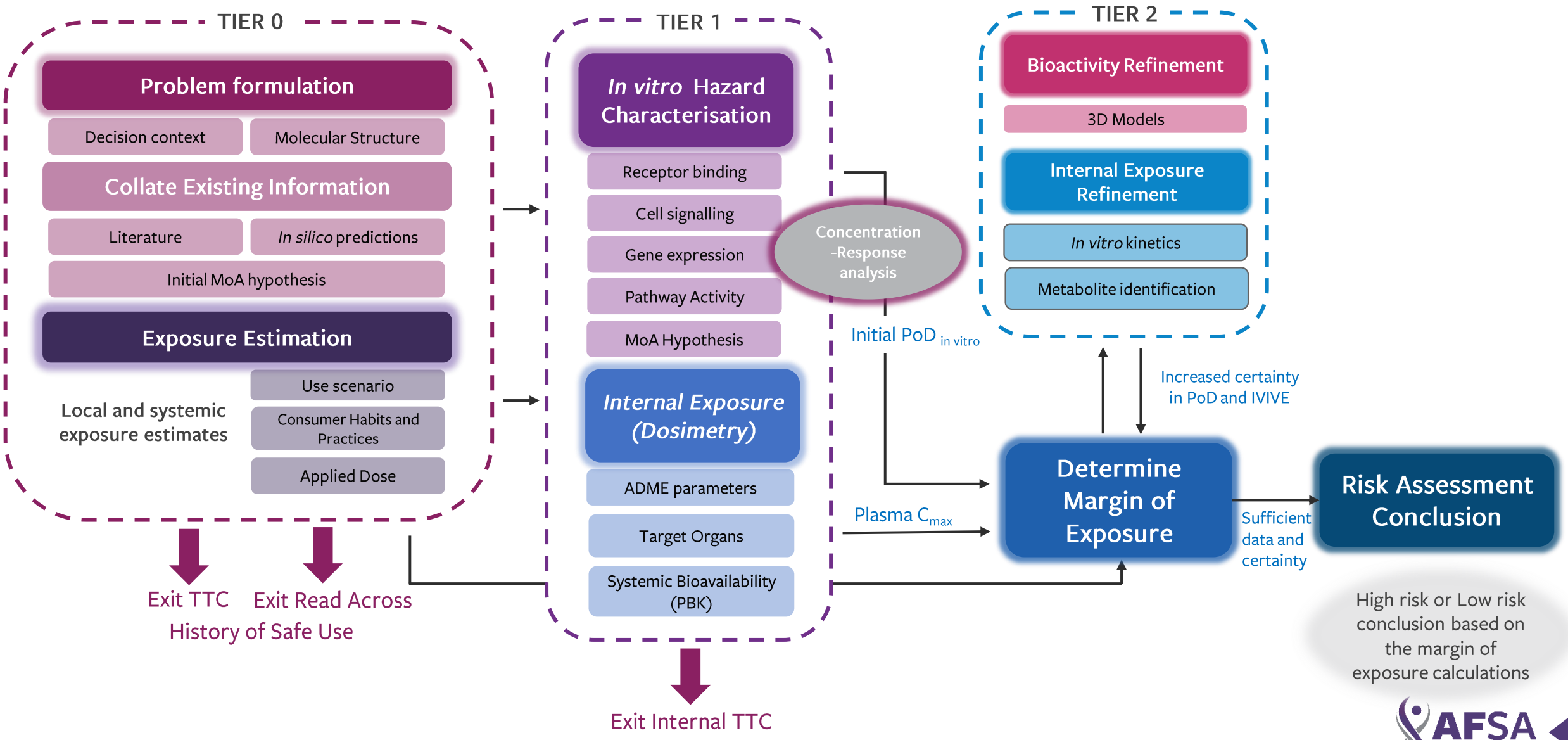
PBPK & IVIVE Application to Risk Assessment

Bringing chemical dosimetry and in vitro bioactivity together to inform risk assessments



Next Generation Risk Assessment (NGRA) Framework



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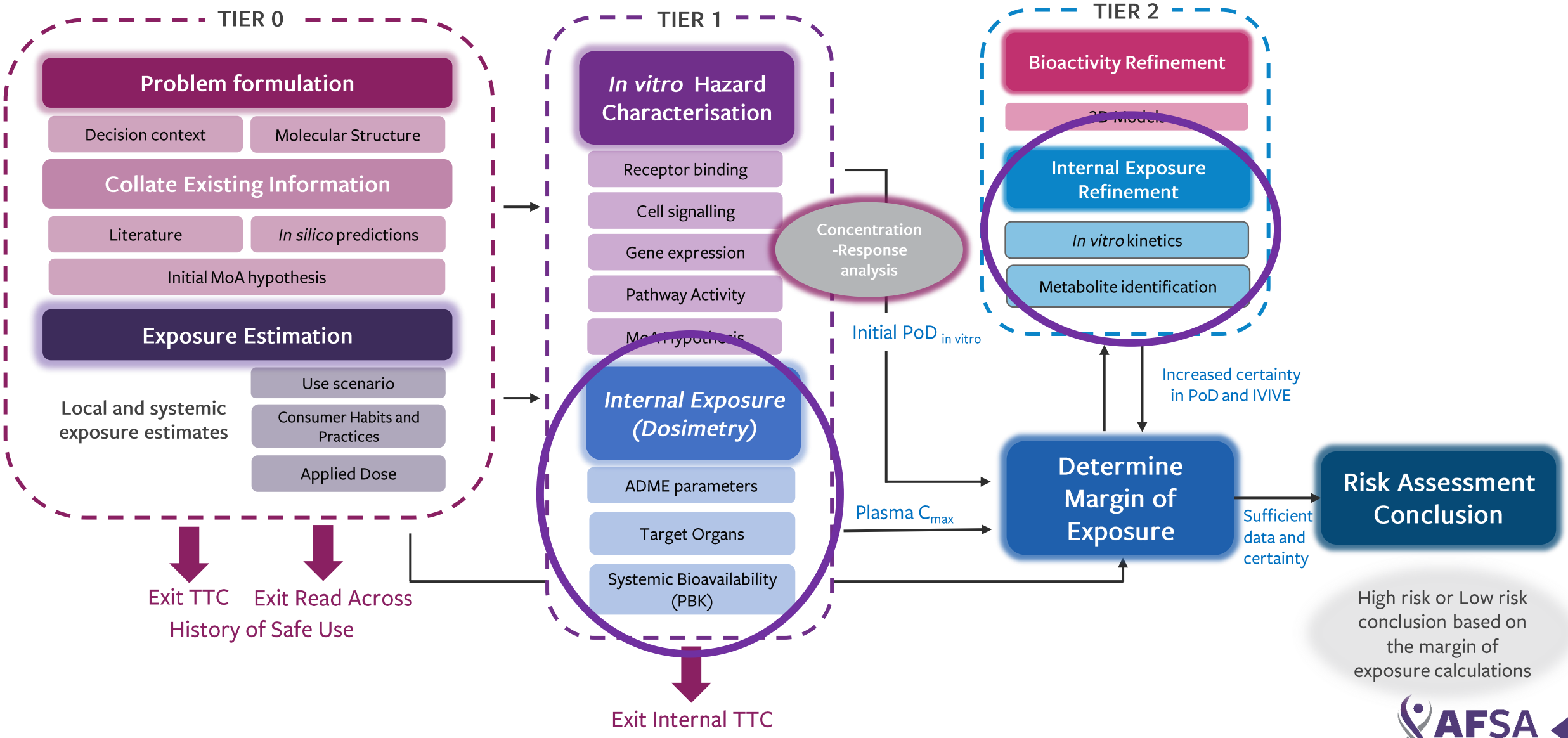
Next Generation Risk Assessment (NGRA) Framework



Thank You!

Allison Schafer Schafer.ae@pg.com
Rebecca Clewell rclewell@21stcenturytox.com

We value your feedback! As the AFSA Collaboration works to complete its free Master Class on Animal-Free Cosmetic Safety Assessment, we would appreciate your input on what we've developed so far and presented via this webinar preview series. Please take our FEEDBACK SURVEY

Definitions

- ADME – absorption, disposition, metabolism, excretion – the processes that determine internal dose of a chemical
- Bioavailability – fraction of administered dose that enters systemic circulation
- Biodynamics - the formal study of vital forces, physiological interactions and behaviors in living organisms. The study of the effects of physical changes and mechanics on biological systems.
- Biokinetics - the study of the metabolism and transport of drugs (pharmacokinetics) or chemicals (toxicokinetics) through the body
  - → Aka dosimetry, chemical disposition
- Free concentration – concentration of chemical in blood that is not bound to proteins
- HPGL - hepatocytes per gram of liver
- HT-IVIVE – high throughput IVIVE – simplified IVIVE model for rapid estimation of in vivo dose with a minimum of measured parameters.
- IVIVE – in vitro to in vivo extrapolation – the process of predicting in vivo exposures from in vitro points of departure
- PBK models – physiologically based kinetic models – models of chemical kinetics that incorporate physiology of the system to allow scalability and extrapolation
- PBPK models – physiologically based pharmacokinetic models – same as PBK models, except that the “pharma” may be thought to imply that chemical has pharmaceutical properties (though PBPK is often used generically for all types of chemicals)
- PBTK models– physiologically based toxicokinetic models – same as PBK models, except that the “toxic” may be thought to imply that chemical has toxic properties
- Point of departure - the dose required for a particular tissue to have an effect
- Q-IVIVE – quantitative IVIVE- generally used to describe an IVIVE model that incorporates more complex process than the standard HT-IVIVE approach in order to refine the estimate of in vivo dose
- Tissue dose – the amount of chemical (in active form) that reaches the target tissue.

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