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Trust Your Gut:
Establishing Confidence in Gastrointestinal Models

National Institutes of Health
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Using Caco-2 Permeability to Estimate Oral Bioavailability for Environmental Chemicals

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The authors declare no conflict of interest

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Outline

- Bioavailability in context
- Caco-2 Assay Method
- Data Analysis
- Data Utility

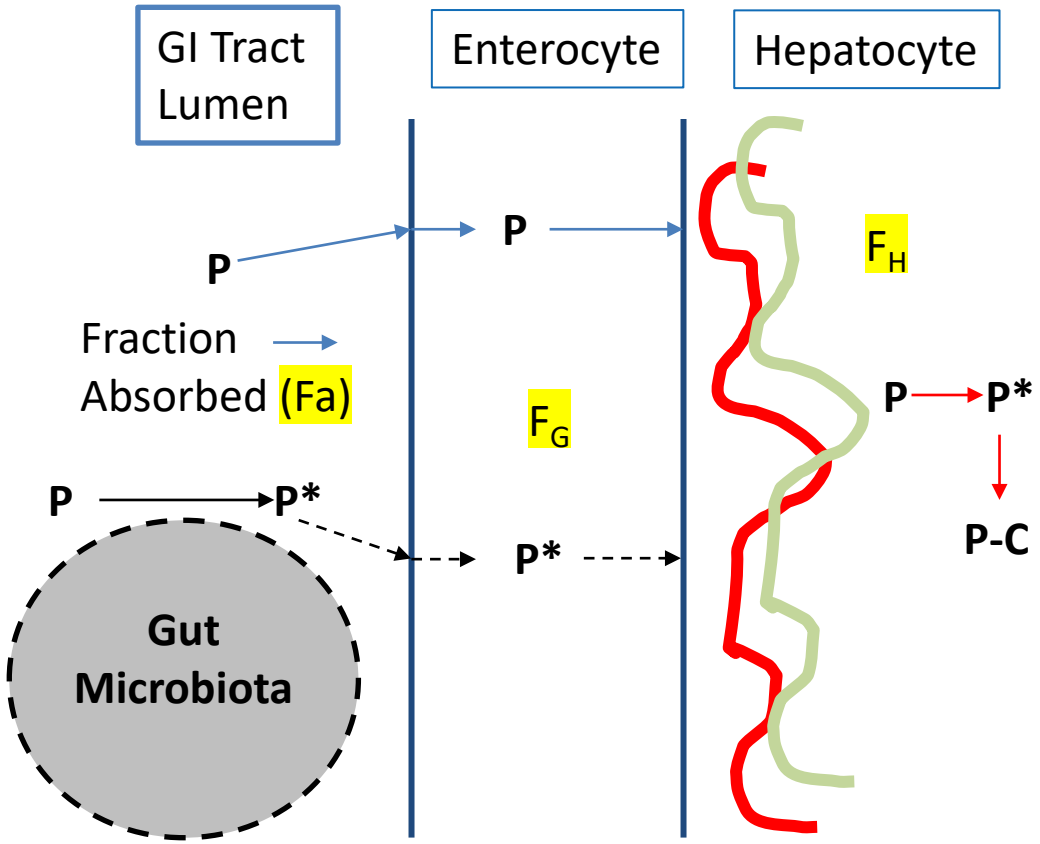
What is Oral Bioavailability?

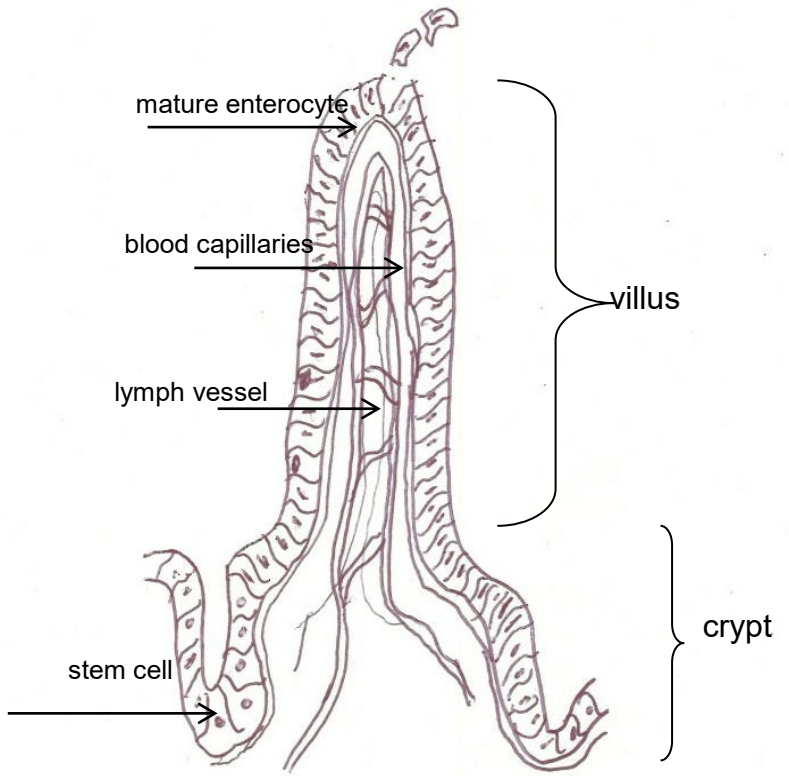
- Rate and extent of chemical absorption into the systemic circulation

$$F_{oral} = (F_a)\,(F_G)\,(F_H) = (F_a)(1-E_G)(1-E_H)$$

F_a – fraction of dose absorbed into GI tract wall,
F_G – fraction of dose escaping first pass elimination in the GI tract wall
F_H – fraction of dose escaping first pass elimination in the liver
E_G – GI first pass extraction ratio
E_H – Hepatic first pass extraction ratio

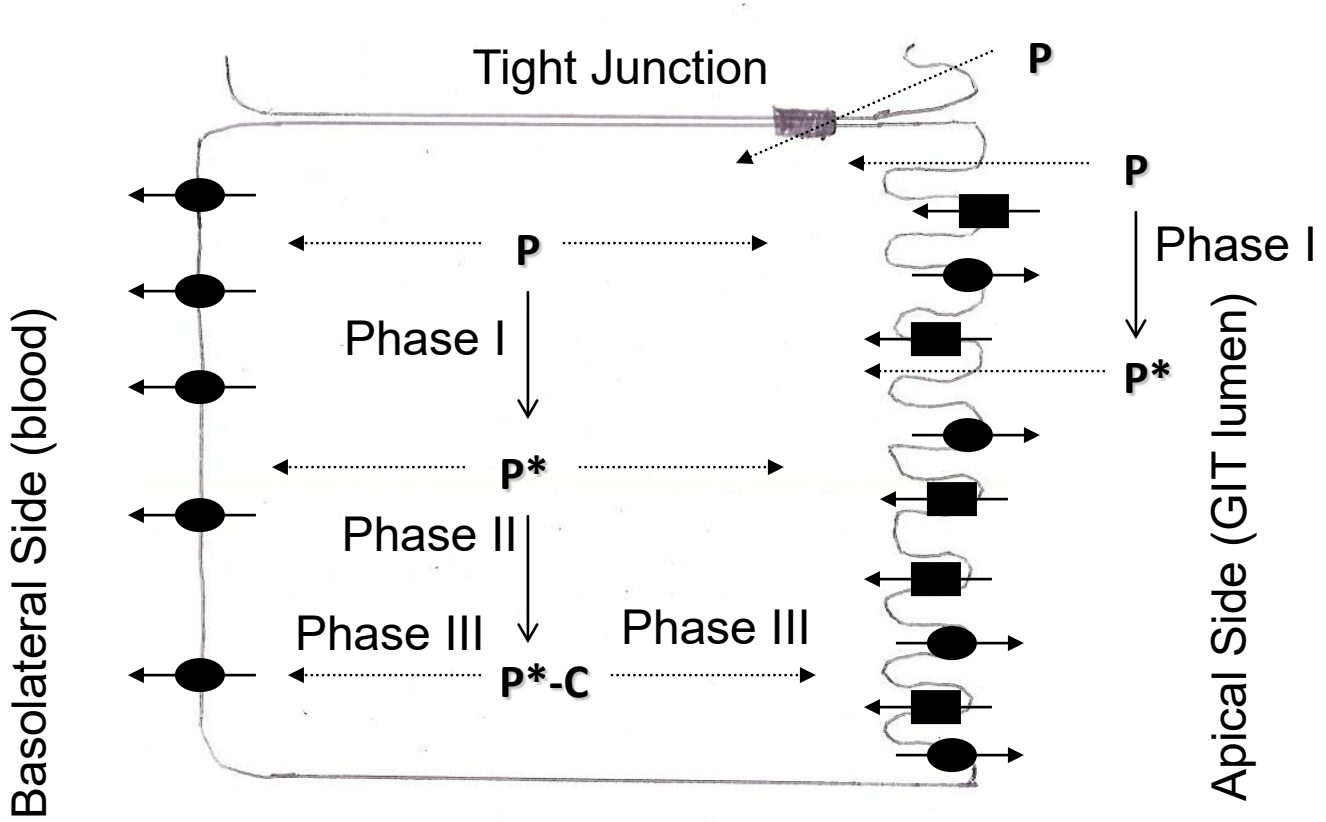
Bioavailability





P is parent chemical, P* is product of Phase I metabolism. P*-C is conjugated product of phase II metabolism

Biological Systems are Dynamic



- Passive Diffusion
  - Paracellular
  - Transcellular
- Active Transport
  - Either direction
- Biotransformation
  - Gut Microbiota
  - Enterocyte
  - Hepatocyte
- Gut function
- Host Factors

P is parent chemical, P* is product of Phase I metabolism. P*-C is conjugated product of phase II metabolism

How to Assess Oral Bioavailability

In Vivo

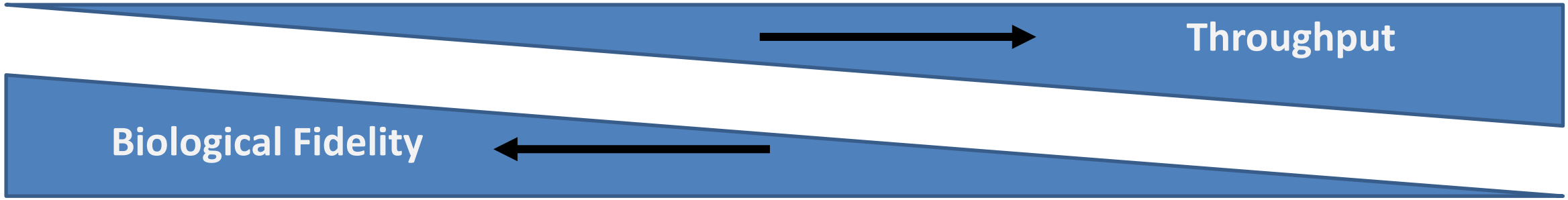
- Oral & iv dosing with measurement of time course
- Intestinal perfusion techniques

In Vitro

- Non cellular bioaccessibility measures
- 2D synthetic membranes
- 2D cellular models (e.g., Caco-2)
- 3D cellular models
- 3D fluid dynamic systems

In Silico

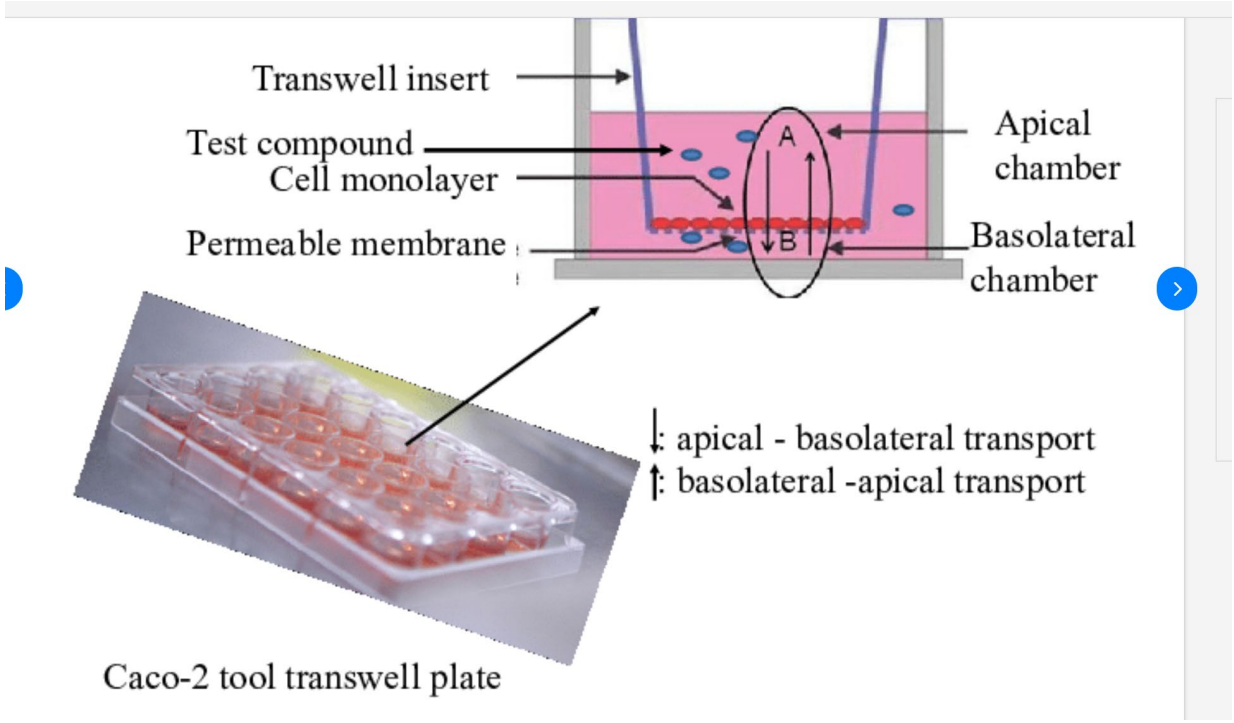
- Predictive QSAR or QSPR models
- Chemical-specific PBPK models
- Complex multi-compartment gut models



Why Caco-2 Cells?

- Commercially available and well characterized
- Widely used in pharmaceutical industry (more data publicly available)
- Can mimic many structural and biochemical features of human intestine
- Apparent permeability (P_{app}) measured in Caco-2 cells correlates with effective permeability rate (P_{eff}) *in vivo* (F_a estimation)
- Some Limitations/Concerns
  - Absence of some GI tract features
  - Transporter and metabolizing enzyme expression can vary
  - Best suited for assessing passive transcellular membrane transfer

Caco-2 Assay

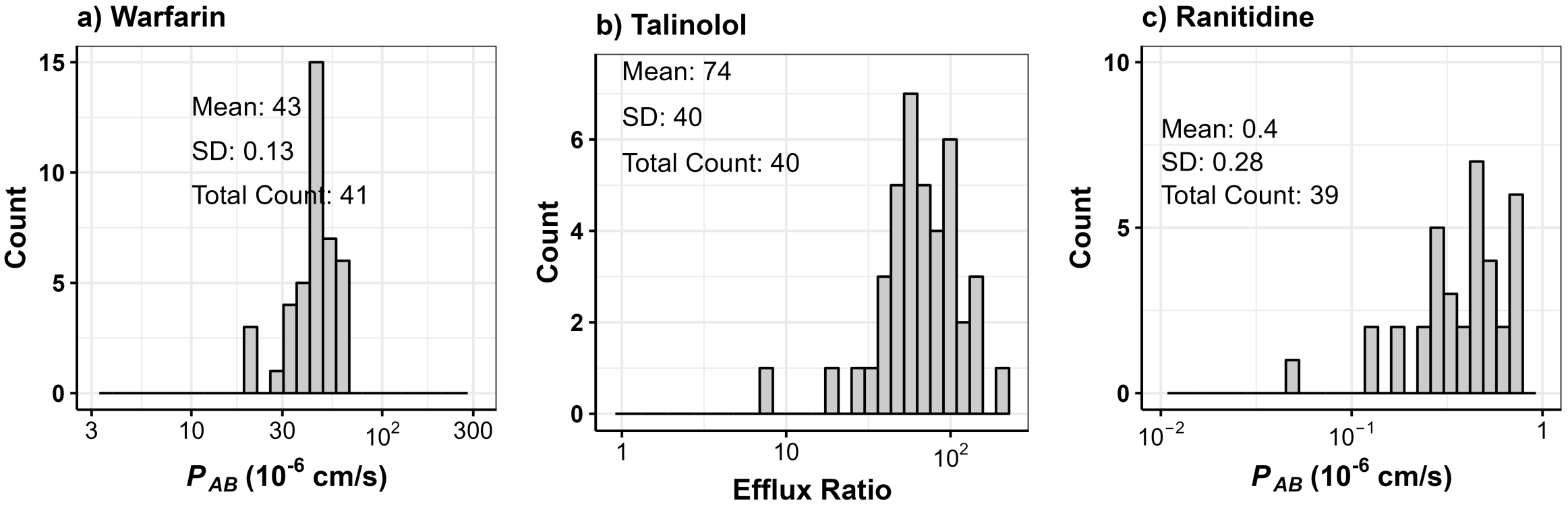


- ToxCast chemical library (n=484)
- Performed at Cyprotex
- Measured P_{app}^{AB} & P_{app}^{BA}
- Calculated of Efflux Ratio (ER)
- Reference chemicals
  - Warfarin (high permeability)
  - Ranitidine (low permeability)
  - Talinolol (P-gp active efflux)
- Monitor barrier integrity
- Analysis by LC-MS/MS or GC-TOF

Caco-2 Data Analysis

- $P_{app} = (dQ/dt) / C_0 \times A$
  - dQ/dt is the rate of passage of the drug across the cells , C_0 is the donor compartment concentration at time zero (derived from analyzing the dosing solution) and A is the area of the cell monolayer
- Permeabilities were calculated based on the ratio of the peak area in the receiver chamber to the peak area of the dosed concentration, normalized by volume
- Fractional recovery ($Frec^{AB}$ and $Frec^{BA}$) was determined by the ratio of the sum of the peak areas for each analyte relative to the C_0
- Permeabilities with corresponding low (< 0.4) or high (> 2) recoveries flagged and not used in subsequent analyses

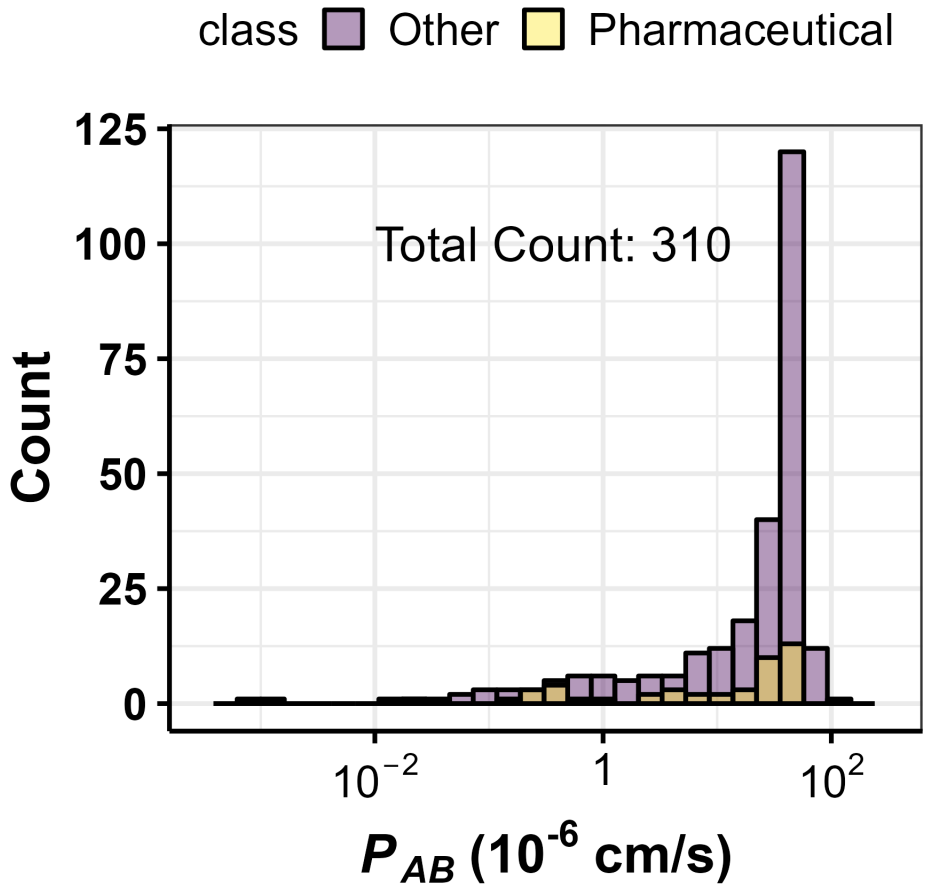
Reference Chemical Data



- Data for reference chemicals used to demonstrate that assay is working as expected
- Warfarin (high permeability), Talinolol (active transport), Ranitidine (low permeability)
- Reference chemical measurements provide ~40 sets of duplicates (2 measured on each plate) and variability of the reference chemicals is similar to test chemicals which were assayed once in duplicate

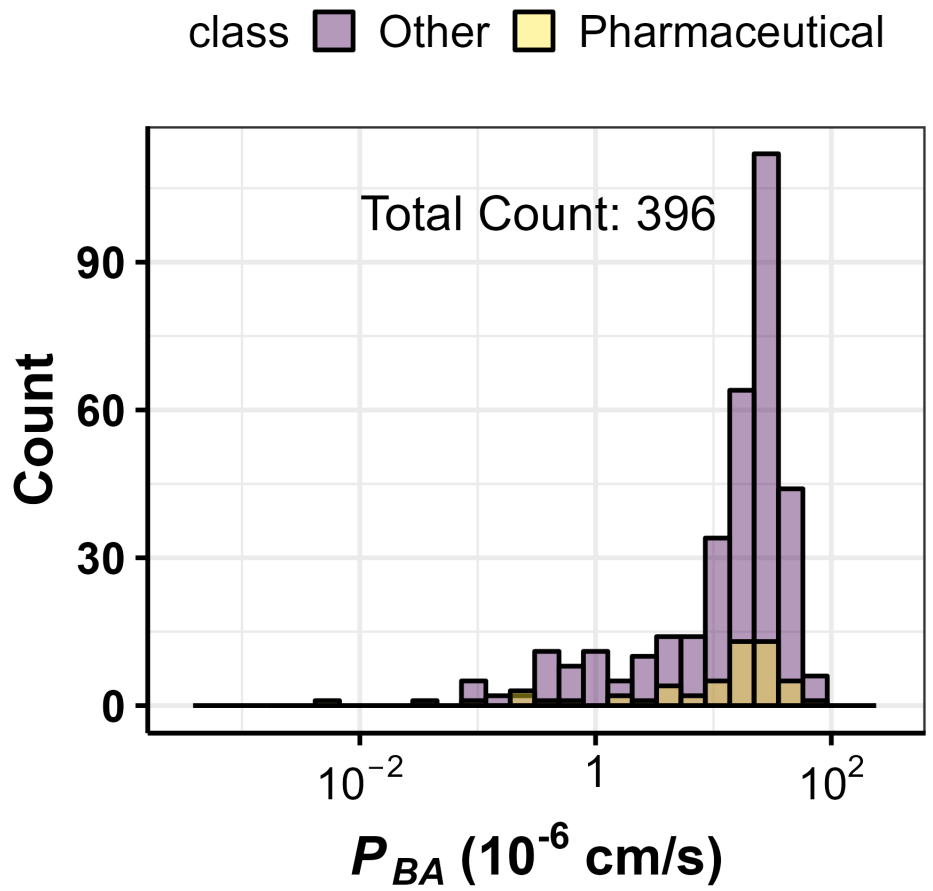
Apical to Basolateral (P_{app}^{AB})

- Equivalent to gut lumen side
- P_{app}^{AB} measurable for 474 of 484
- After filtering (based on F_{rec}), 310 remain
- Generally high permeability
- Data used to:
  - Estimate F_a
  - Develop QSPR model (F_a)
  - Incorporate in httk framework to estimate F_{oral}
  - Evaluate impact on Bioactivity Exposure Ratio (BER)



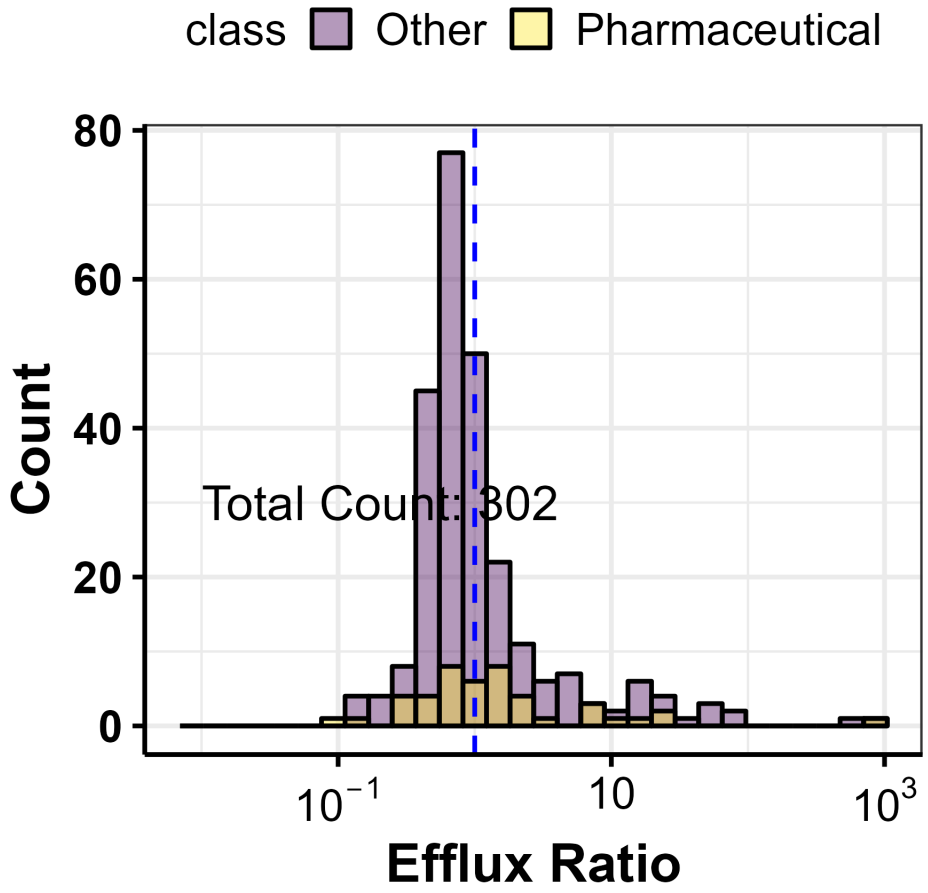
Basolateral to Apical (P_{app}^{BA})

- Approximates to vascular side
- Data used in calculation of Efflux Ratio (ER)
- P_{app}^{BA} measurable for 478 of 484
- After filtering (based on F_{rec}), 396 remain



Efflux Ratio (ER)

- Calculated from measured P_{app} values
- ER = $P_{app}^{BA}/P_{app}^{AB}$
- Calculated for 302 chemicals
- ER > 2 suggests active transport

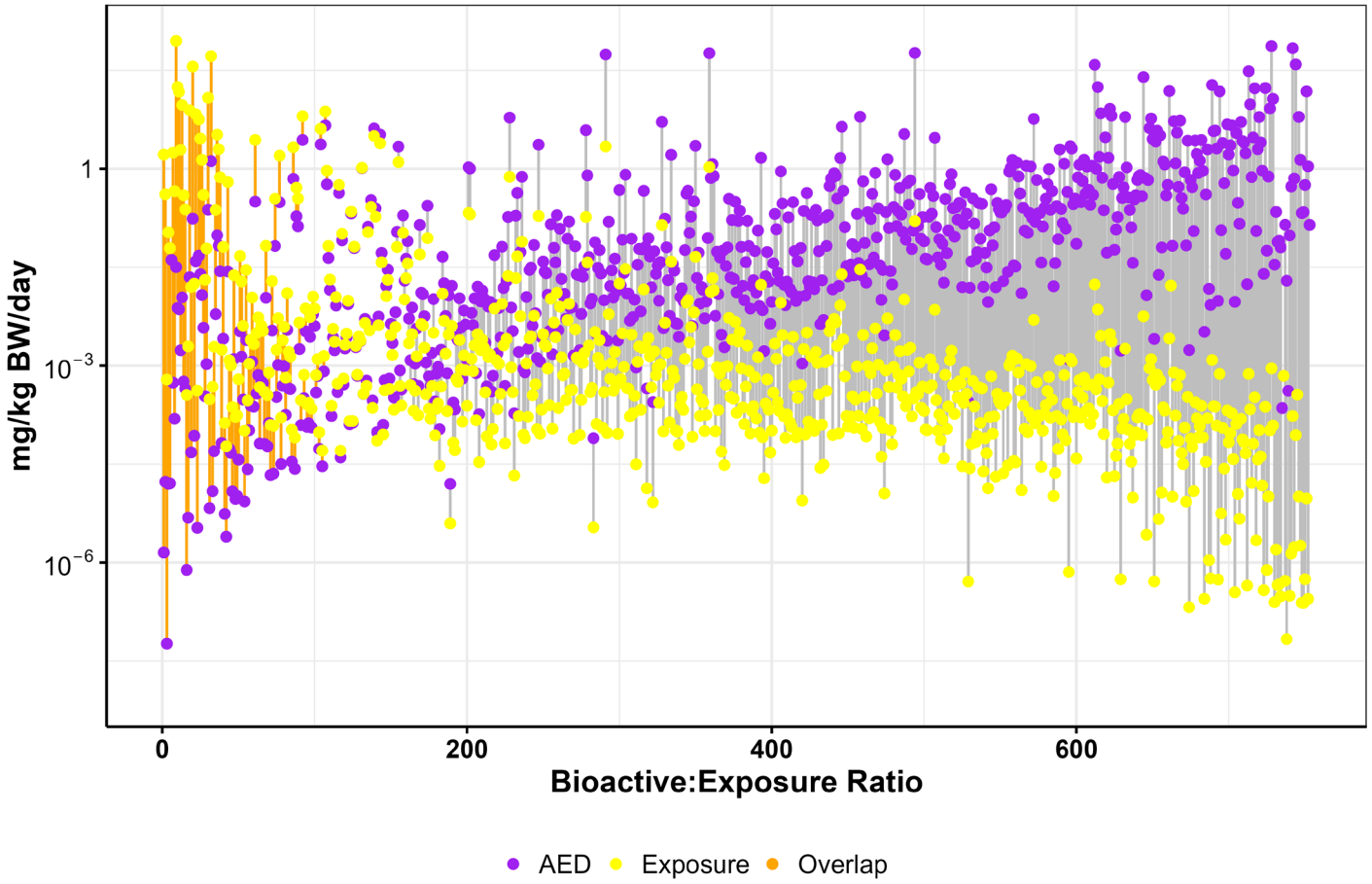


Caco-2 Data Utility

- Comparison to pharmaceutical data
- Variability reference chemical data
- Estimation fraction absorbed (Fa)
- Building QSPR and other predictive models
- Incorporation into existing frameworks (e.g., httk) to provide refined estimates bioavailability
- Impact of refined estimates of bioavailability on screening level risk prioritizations
- Soon to be publicly available via httk R package, Github, and CompTox chemicals dashboard

Implications for Risk Prioritization

- AED (mg/kg/day) estimated based nominal in vitro bioactive concentration
- Exposure (mg/kg/day) is prediction of median U.S. population daily intake rate derived from SEEM3 model
- Overlap indicates higher concern
- Overall reduction in uncertainty when accounting for F_{oral}



AED – Administered Equivalent Dose

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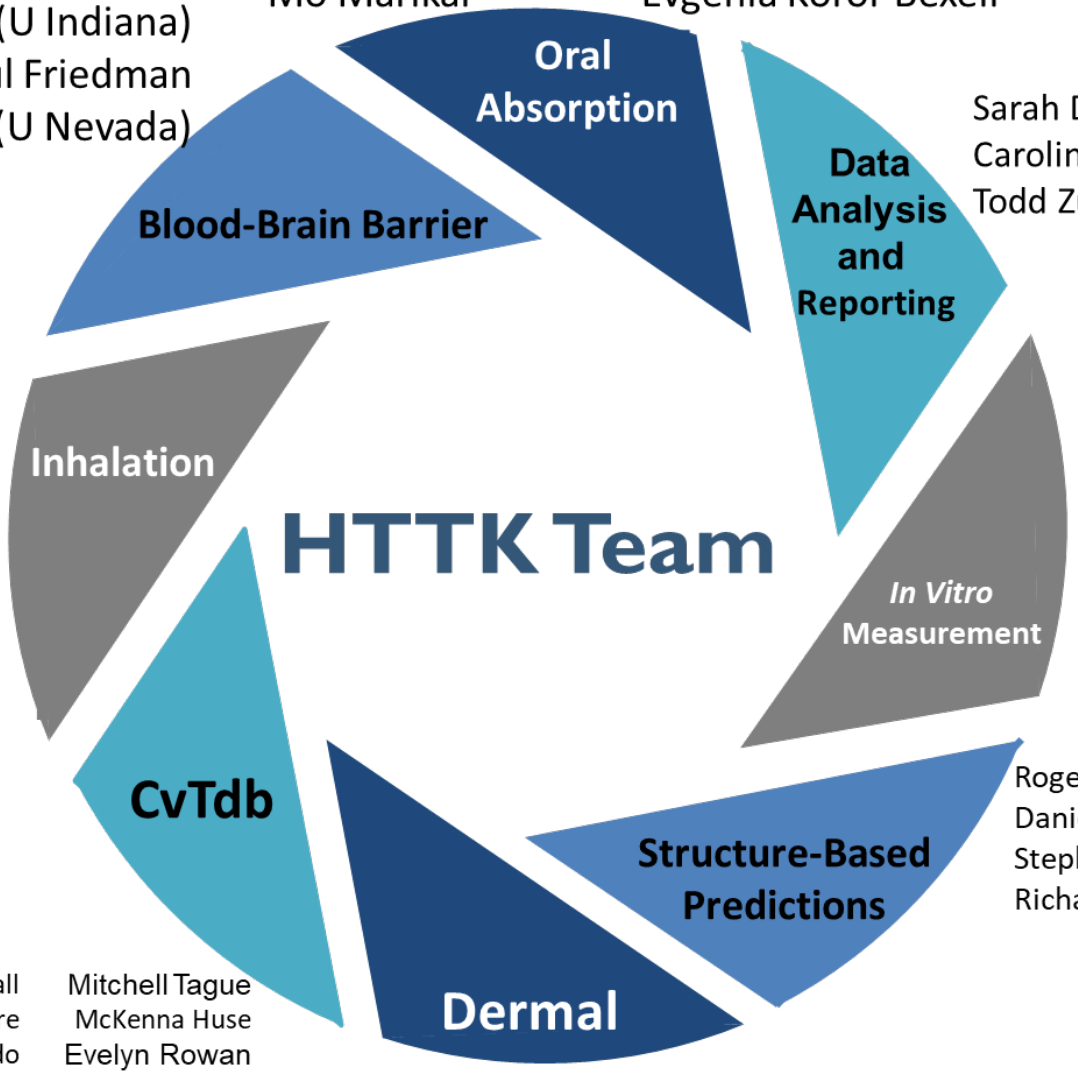

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EXTRA SLIDES

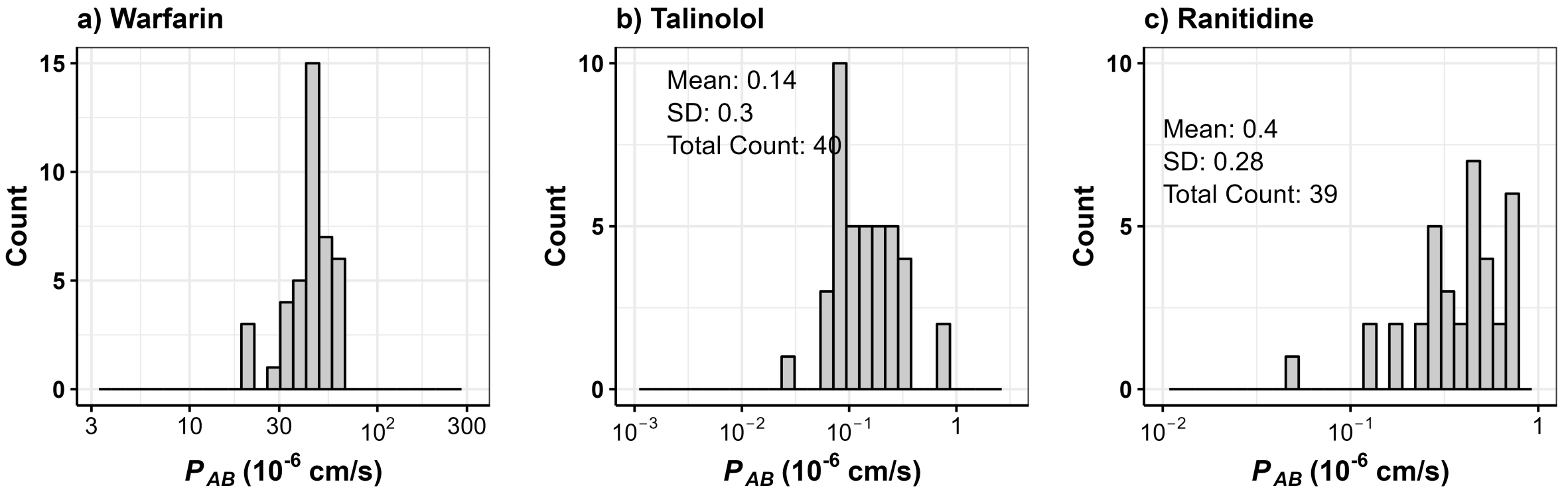
Limitations Caco-2 Assay

- Formula used to calculate permeability
  - $P_{app} = (dQ/dt) / (A * C_0)$
  - Where, dQ/dt is transport rate to receiver side, A is surface area of monolayer, C_0 is initial concentration in doner compartment
- Assumptions
  - Linear accumulation in receiver compartment over time
  - Maintain “sink” conditions in receiver compartment to avoid back diffusion
  - Does not account for cellular accumulation, metabolism, non-specific binding

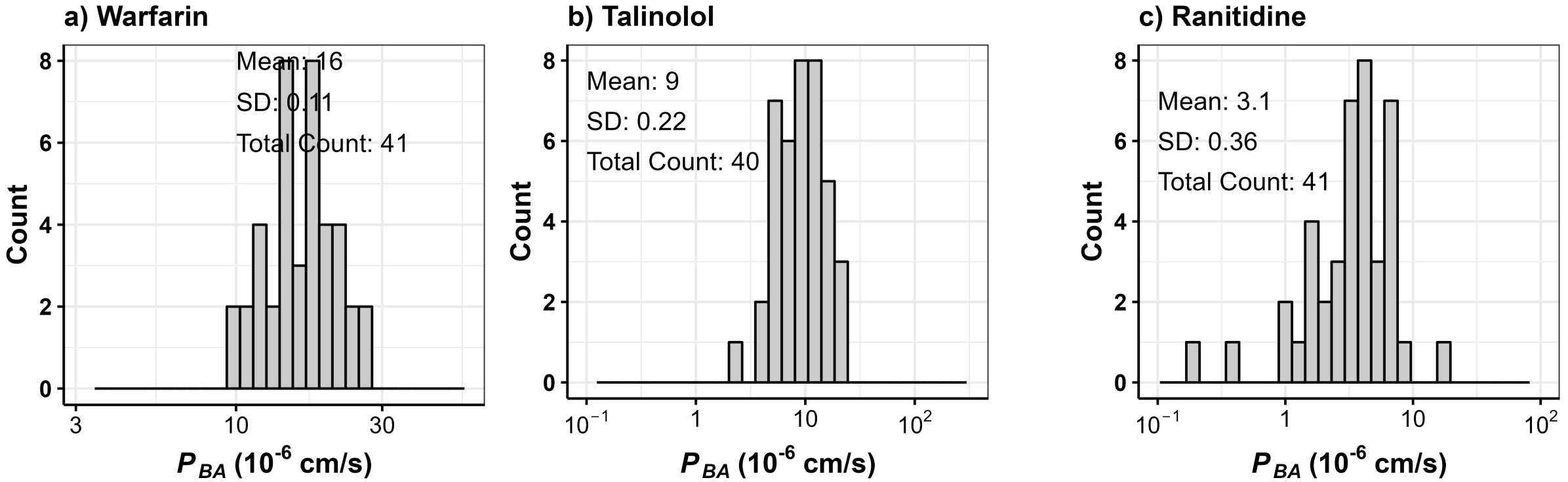
Limitations Caco-2 Assay

- Tight junctions
- Variability in level of protein expression
- Regional differences in protein expression
- Lack of mucous layer
- Inter-laboratory variability

Reference Chemicals - P_{app}^{AB}



Reference Chemicals - P_{app}^{BA}



Reference Chemicals – Efflux Ratio