Evidence from *In Vivo* Skin Stripping Studies:

Utility for Evaluating Topical Bioavailability

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PPP – March 31, 2016

Evidence from *In Vivo* Skin Stripping Studies: Tape

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Skin Stripping: *Brief history*

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In Vivo Correlation Between Stratum Corneum Reservoir Function and Percutaneous Absorption

ANDRE ROUGIER, DIDIER DUPUIS, CLAIRE LOTTE, ROLAND ROGUET , AND HANS SCHAEFER

Department de Biologie, Laboratoires de Recherche Fondamentale de l'Oréal (AR, DD, CL, RR), Aulnay sous Bois, and Centre International de Recherches Dermatologiques (HS), Valbonne, France

  - Chemical applied to hairless rats for 0.5 h (n = 12)
  - Skin stripped at application site with 6 pieces of adhesive tape applied sequentially (n = 6)
  - Excreta (urine/feces) collected for 4 days (n = 6)

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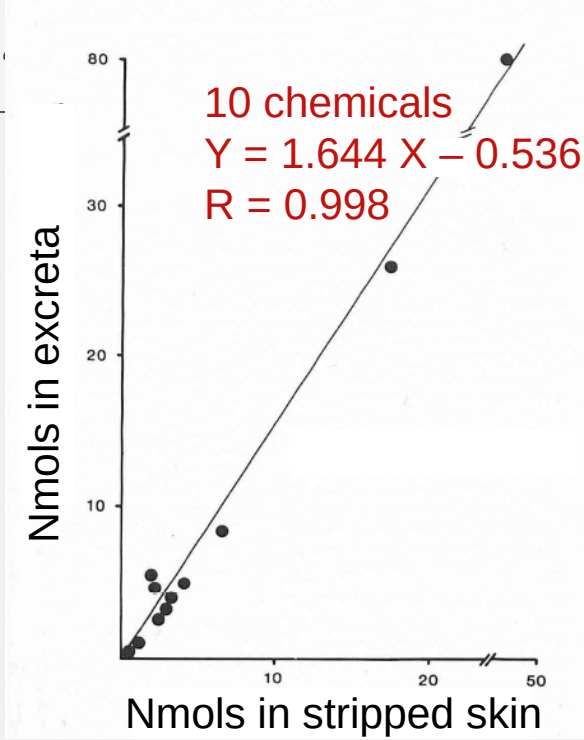
- Chemical applied to hairless rats for 0.5 h (n = 12)
- Skin stripped at application site with 6 pieces of adhesive tape applied sequentially (n = 6)
- Excreta (urine/feces) collected for 4 days (n = 6)
- Chemical amounts in excreta and stripped skin is linearly correlated
- Chemical in skin reservoir absorbs systemically; i.e., it is “available”



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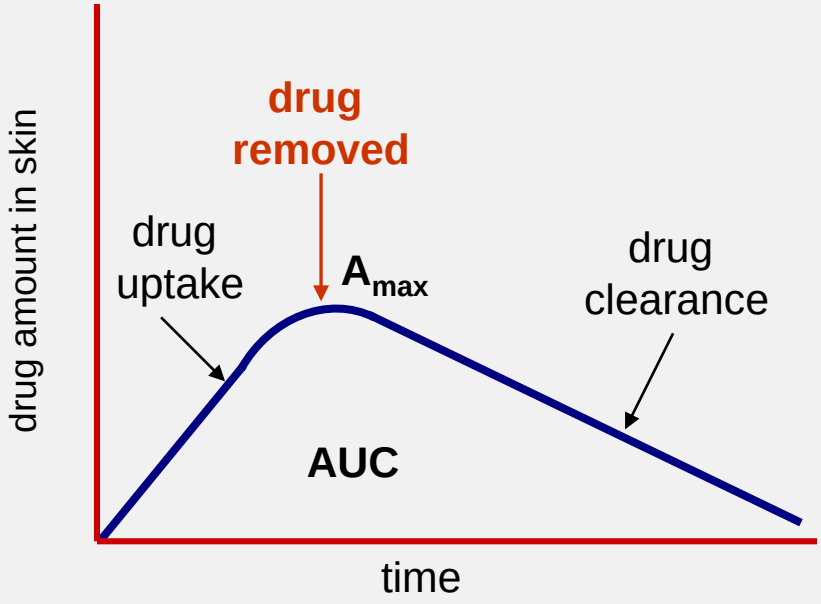
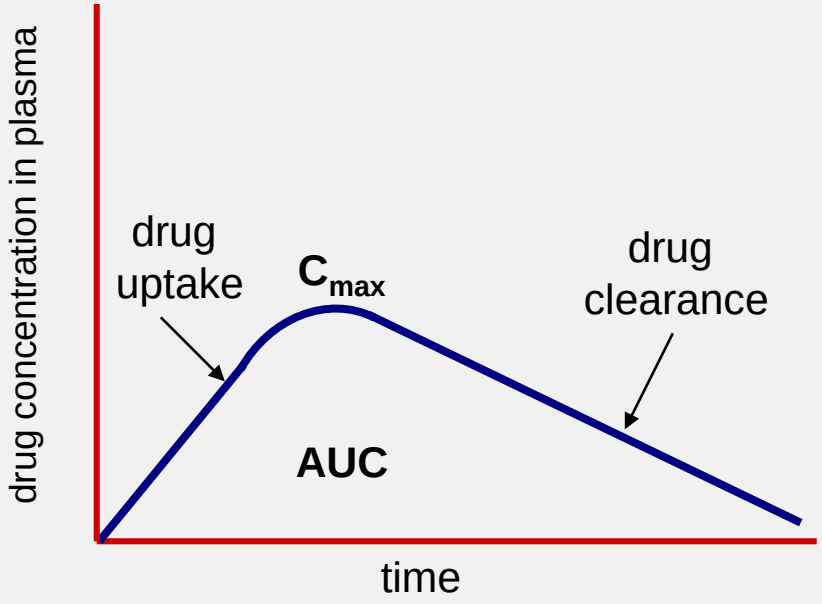
- 1998 – FDA guidance for Dermatopharmacokinetic (DPK) bioequivalence (BE) assessment of topically applied drugs
  - Drug amounts in SC determined in tape stripped skin over time
    - Post drug application (*uptake*)
    - Post drug removal (*clearance*)
  - Drug level vs time characterized by *pharmacokinetic* metrics:
    - Area under drug level-time curve (AUC)
    - Maximum amount (A_{max})
  - BE if AUC and A_{max} are the same

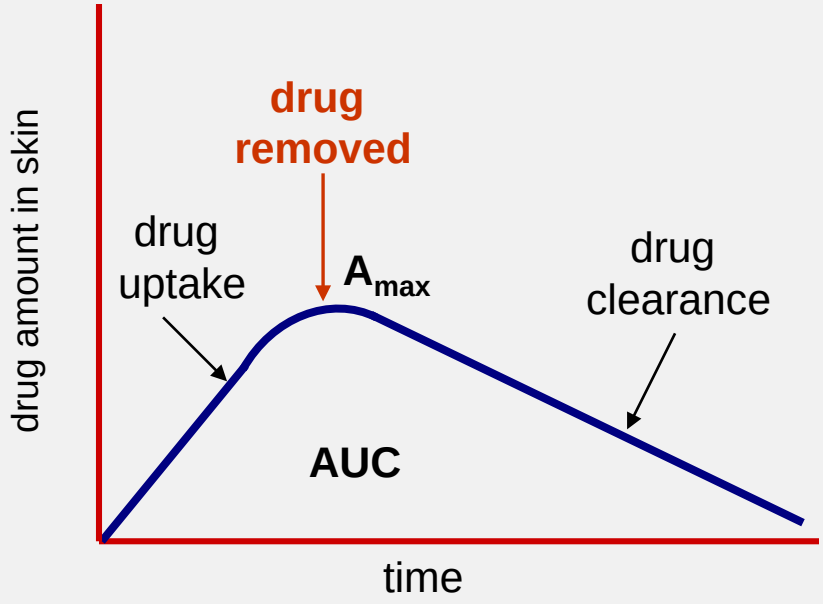


Dermatopharmacokinetics (DPK) for BE test

Similar to pharmacokinetic methods for oral drug assessment



oral drug assessment



topical drug assessment

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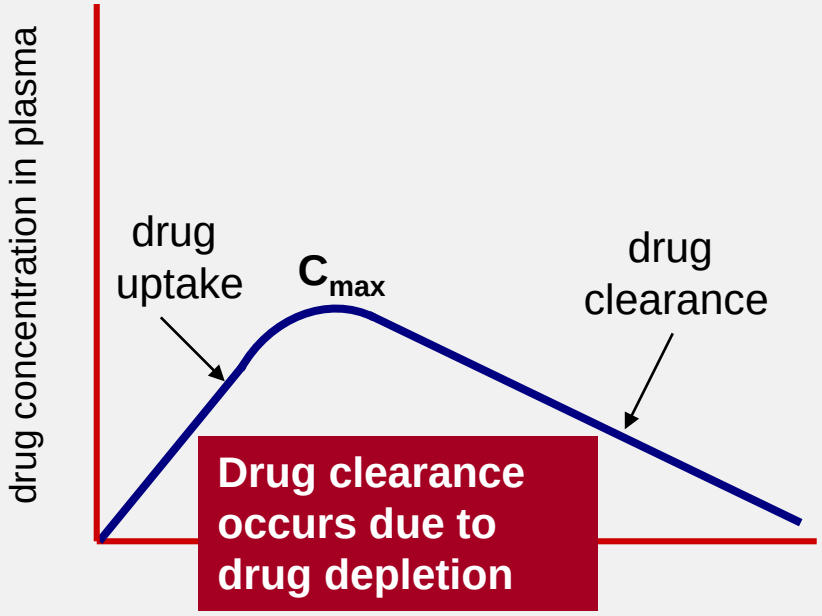

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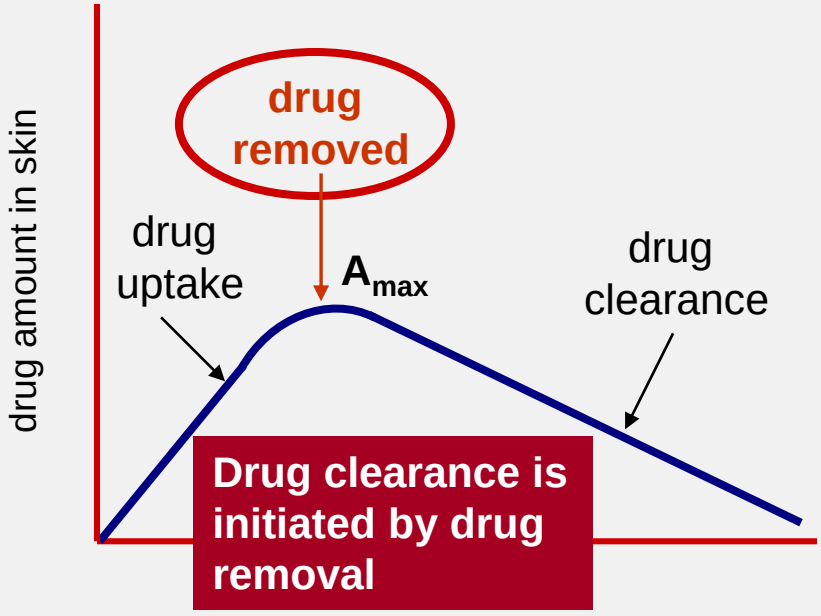

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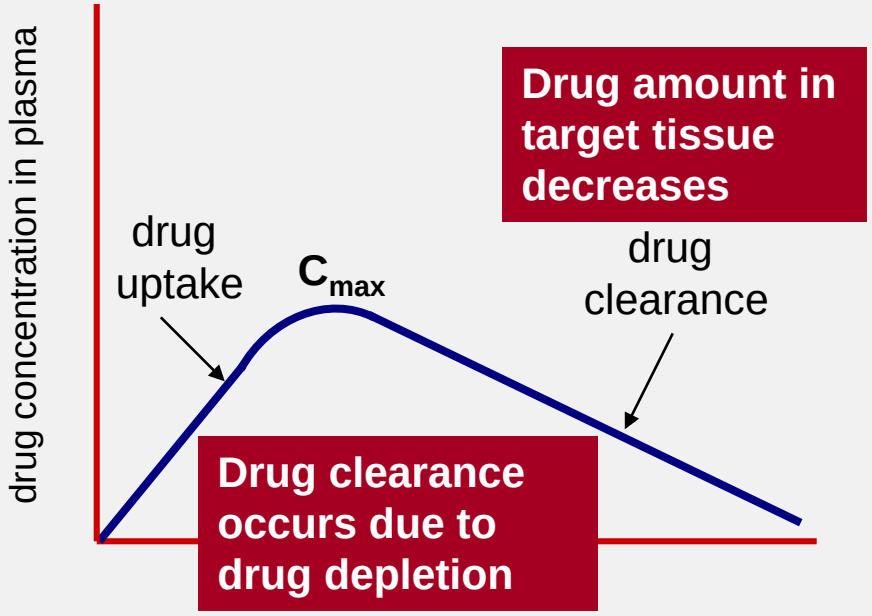

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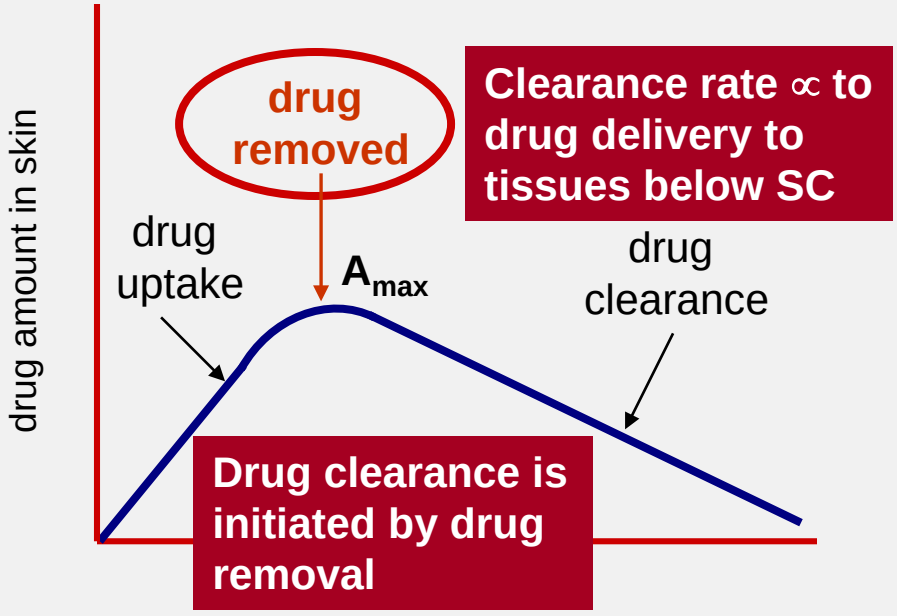
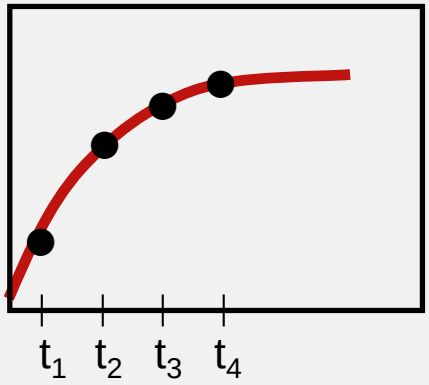


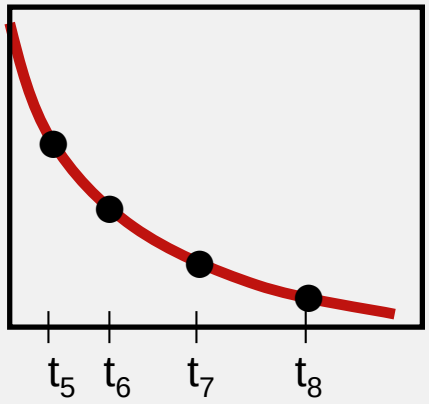
topical drug assessment

DPK BE Study: *0.025% Tretinoin gel products*

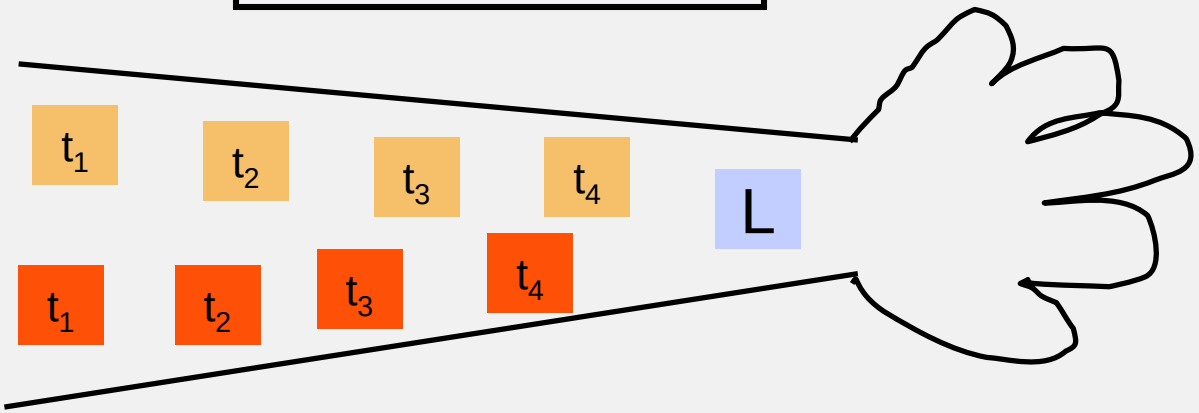
Uptake of active



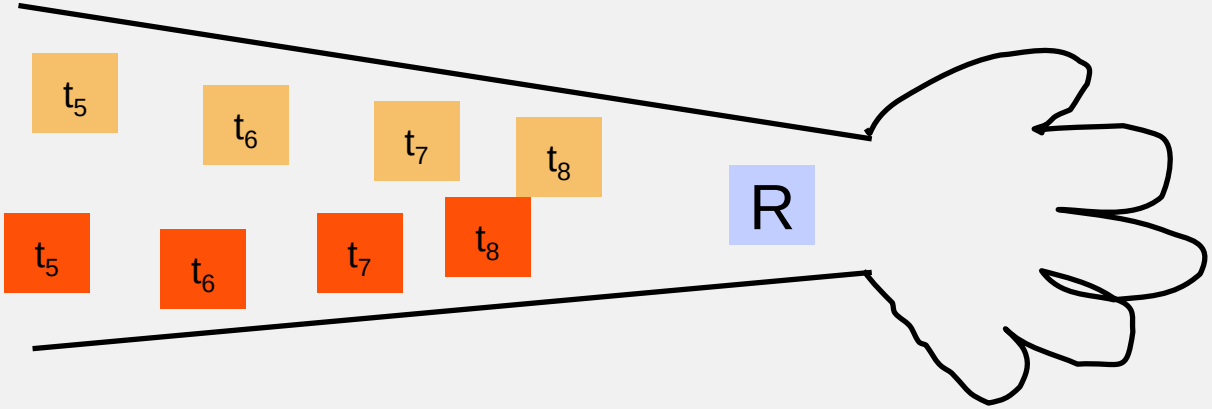
Clearance of active



test versus reference

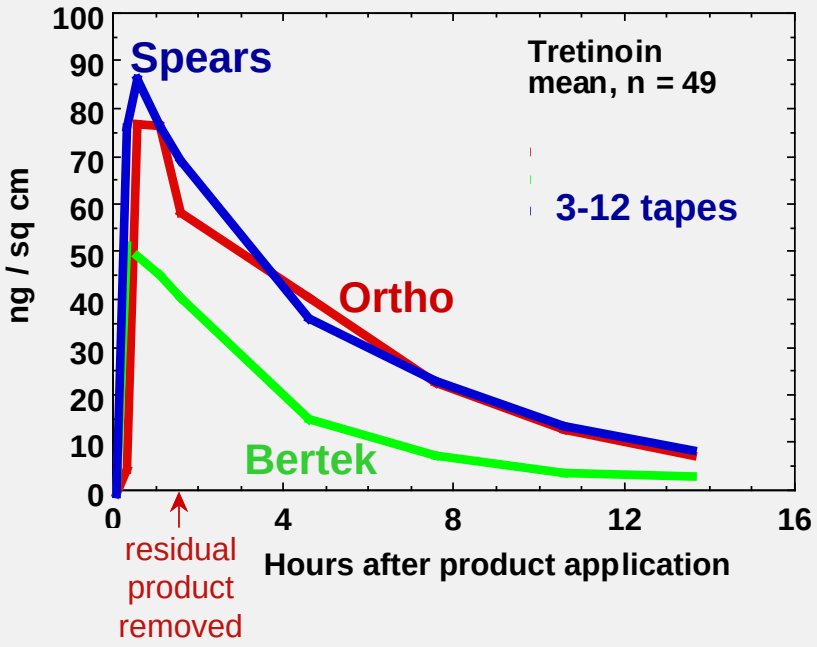


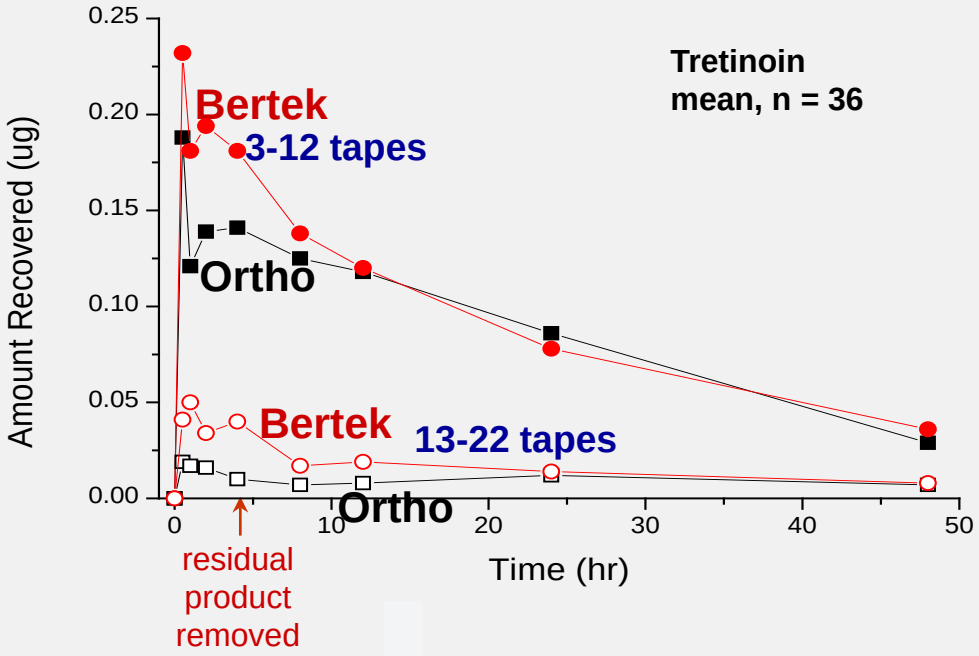
8 sites for each formulation



DPK BE Study: *0.025% Tretinoin gel products*

Ortho = RLD **Spears = Generic (Equivalent)** **Bertek = Inequivalent (less effective)**





Ortho > Bertek

!

Bertek > Ortho

Pershing et al., J Am Acad Dermatol, 2003

Franz, FDA-ACPS, 11/29/2001

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    - 8 sites (time points)/product
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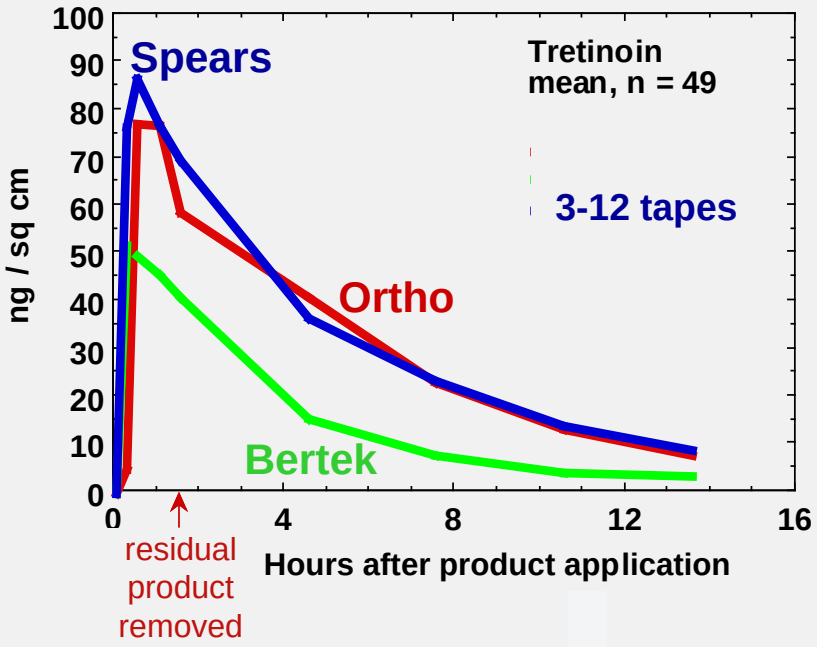

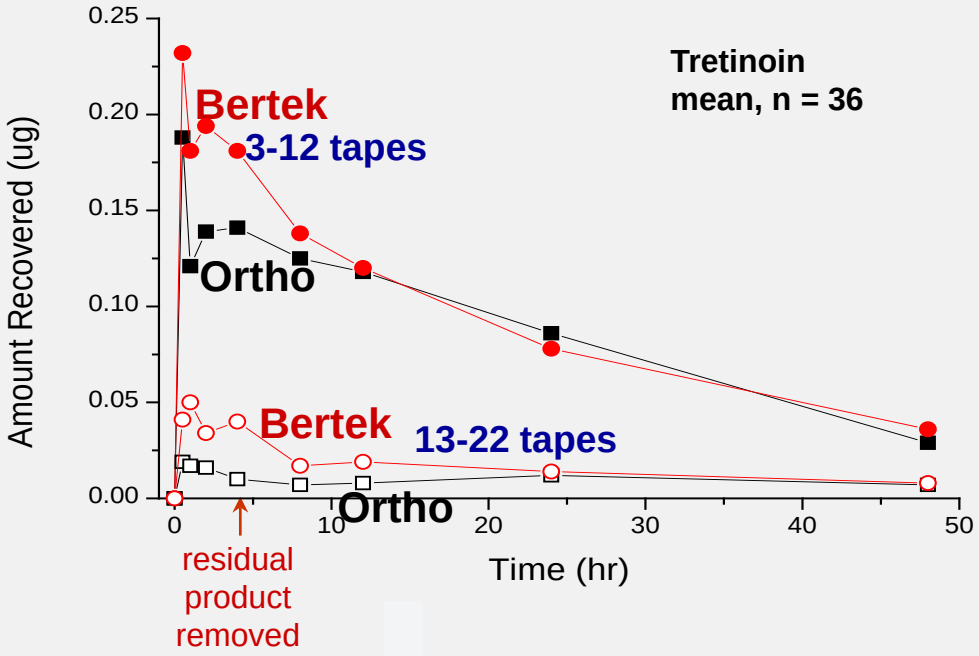

Conner, FDA-ACPS, 11/29/2001

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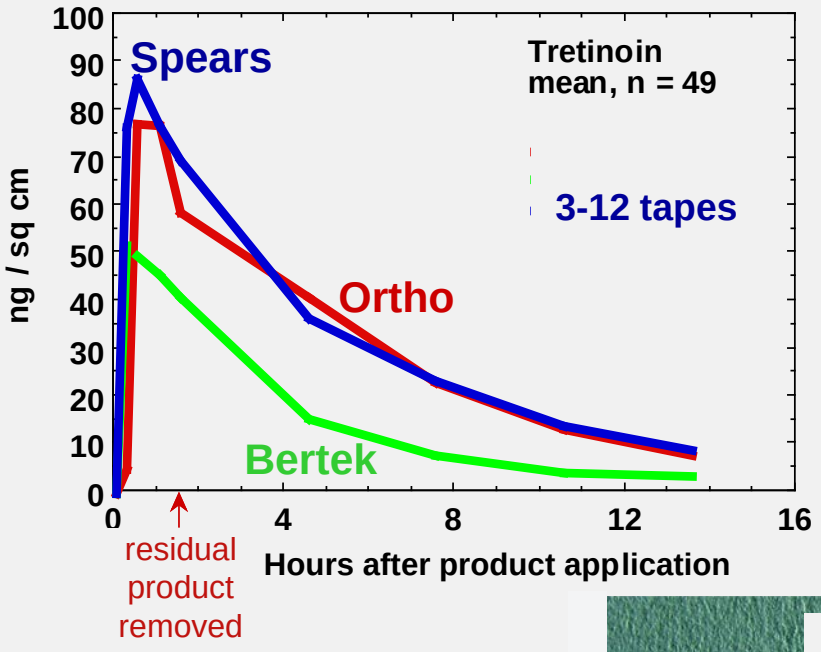

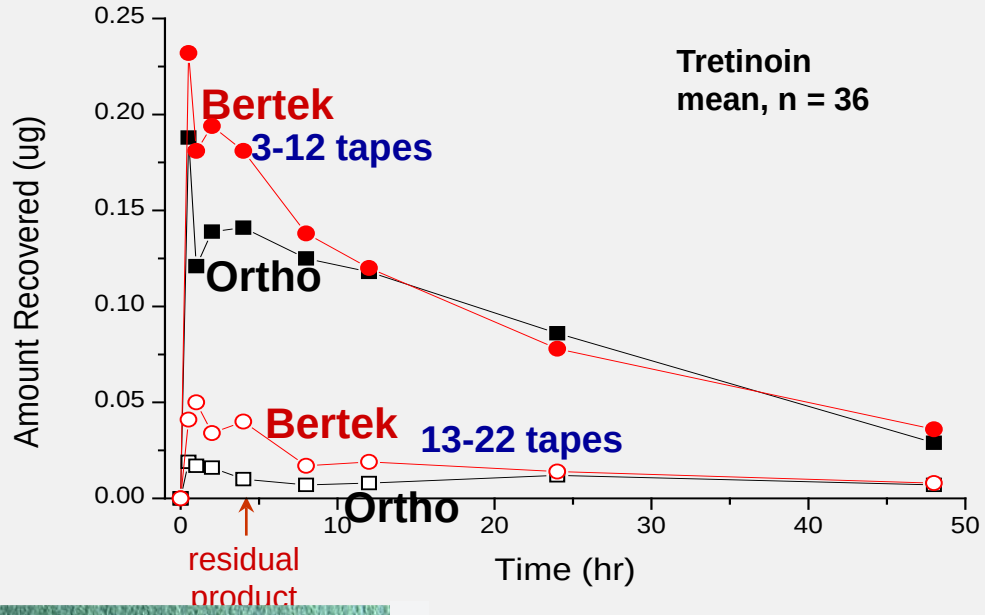

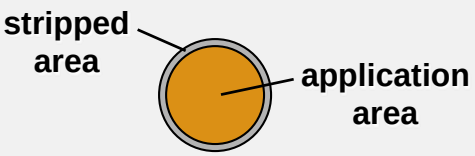

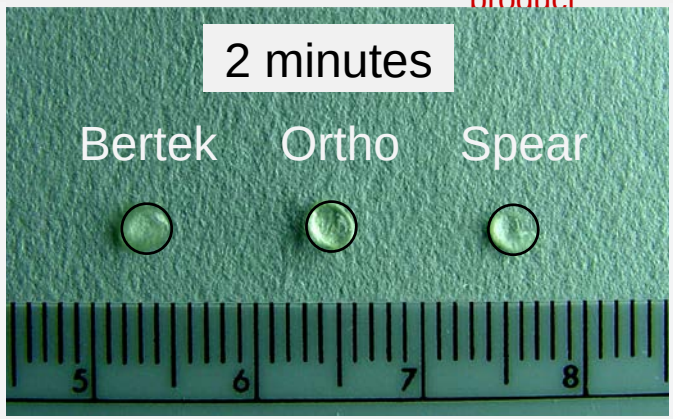

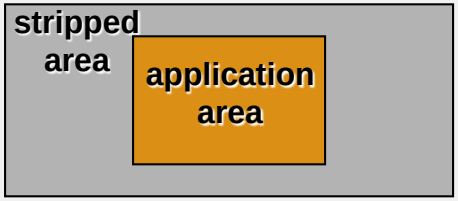

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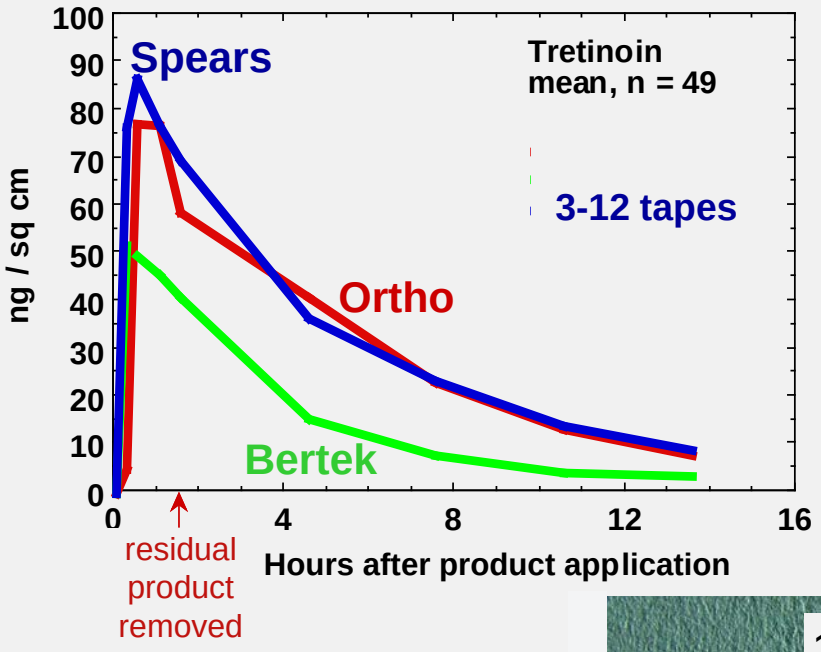

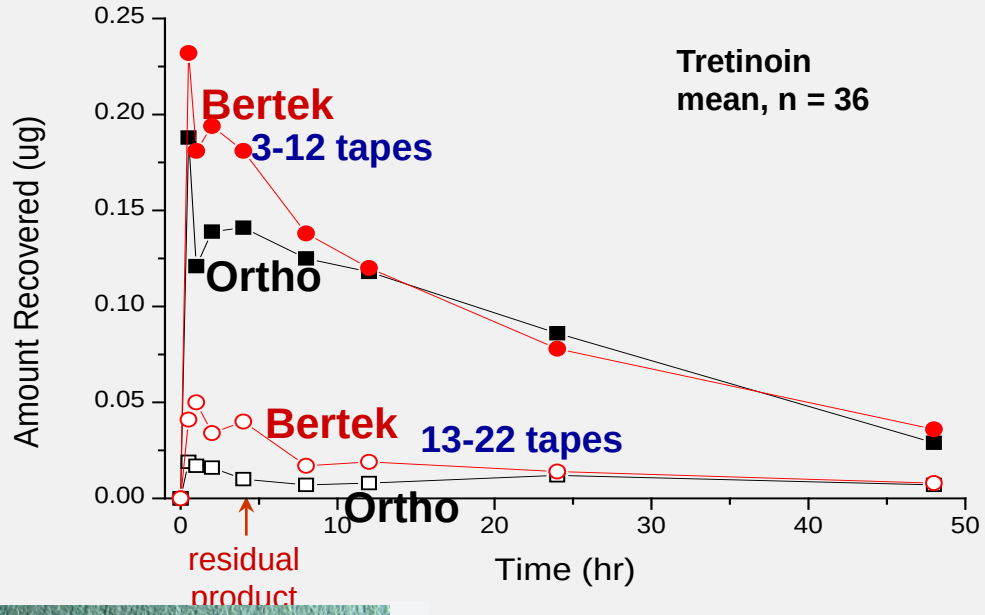

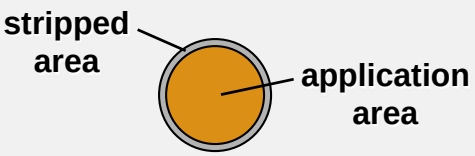

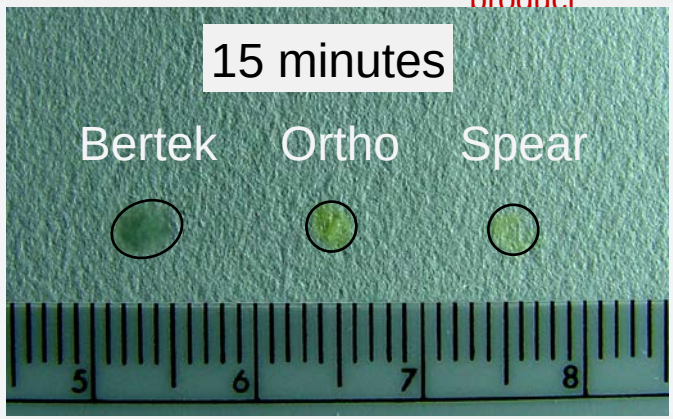

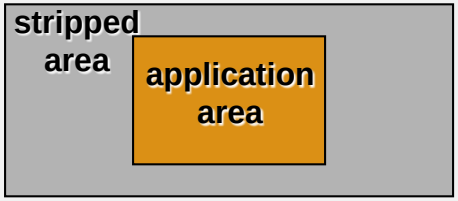

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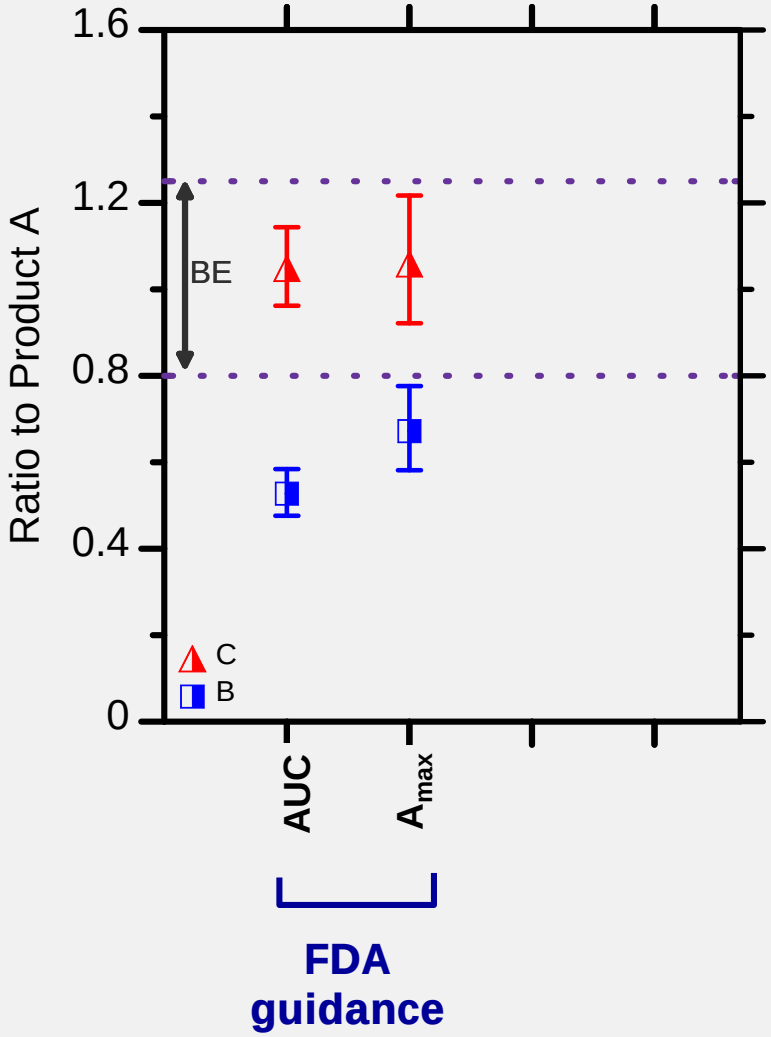
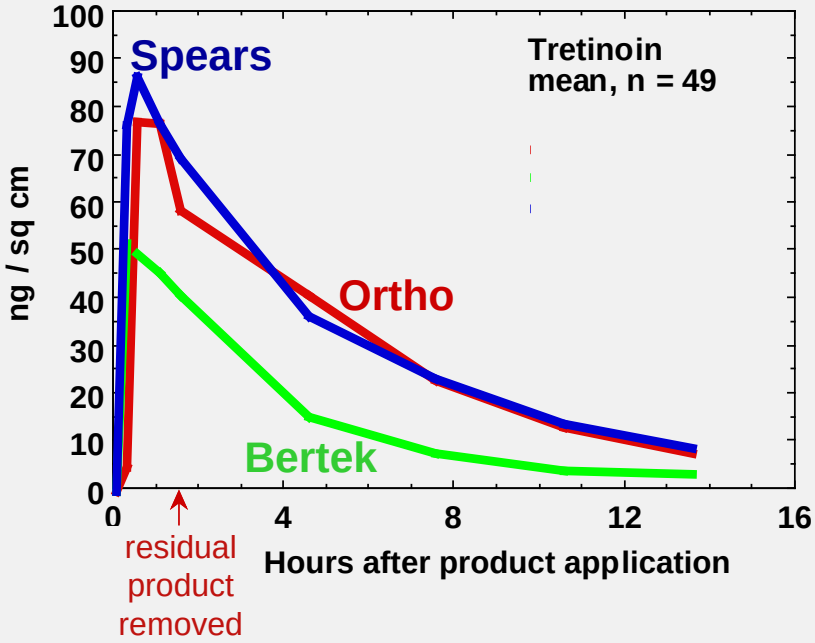
Tretinoin Gel 0.025% Study

Comparing Products B and C to Product A (RLD)





Tretinoin Gel 0.025% Study: *AUC? 8 times?*

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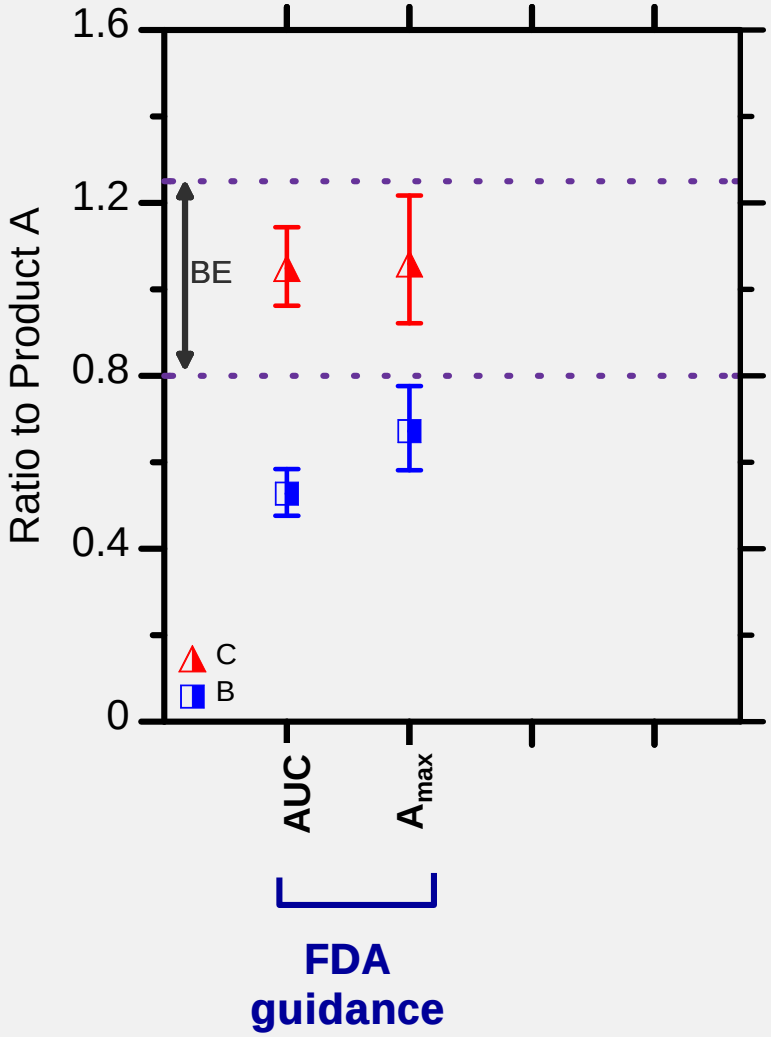

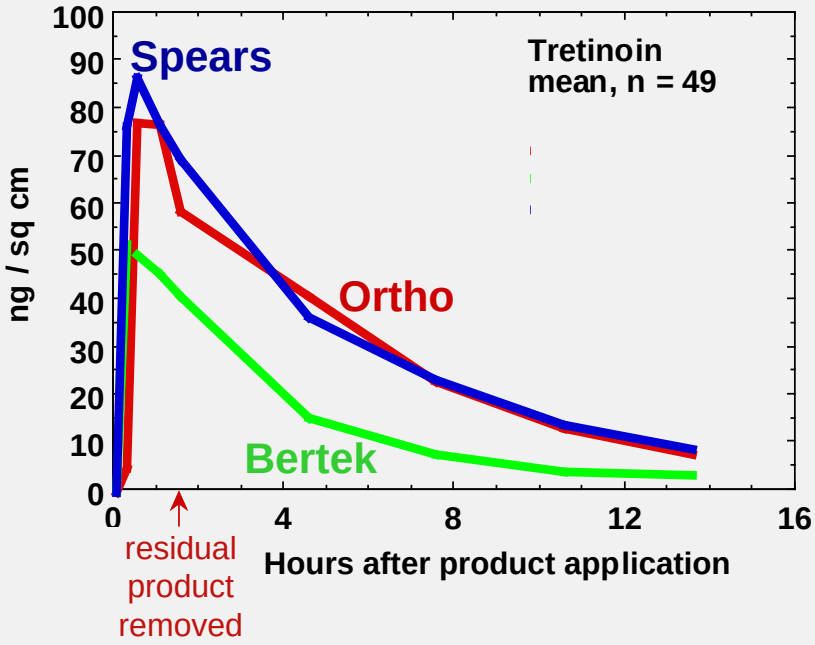

What are the BE ratios at each time point?

Tretinoin Gel 0.025% Study: *AUC? 8 times?*

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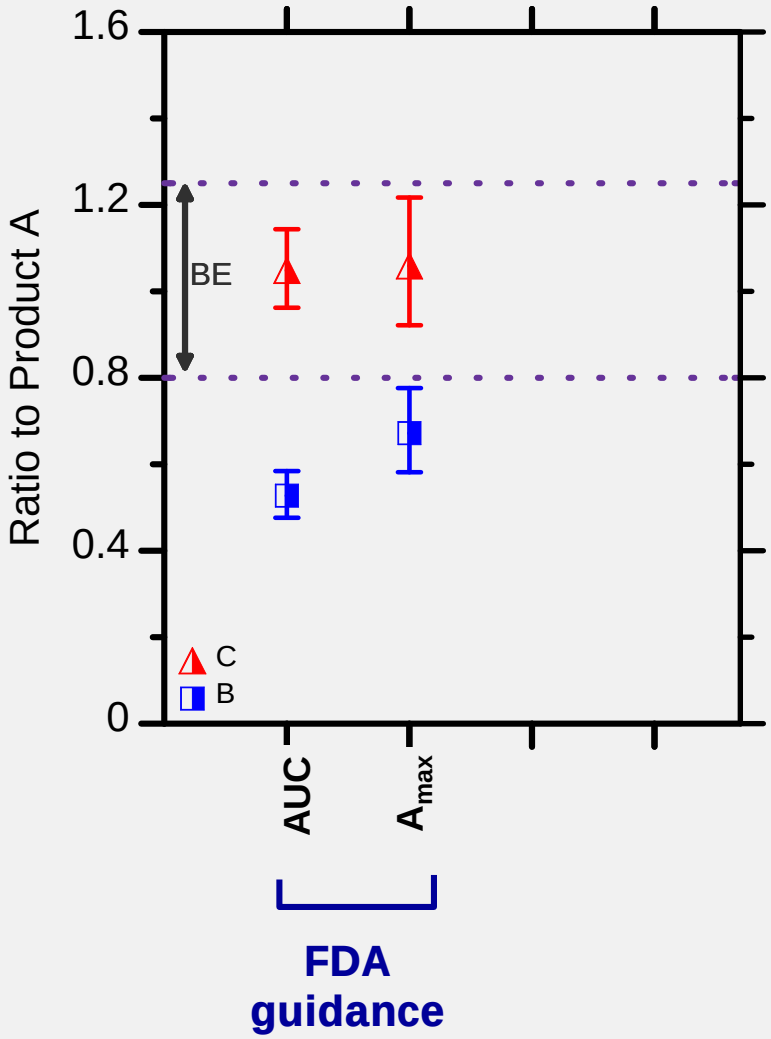

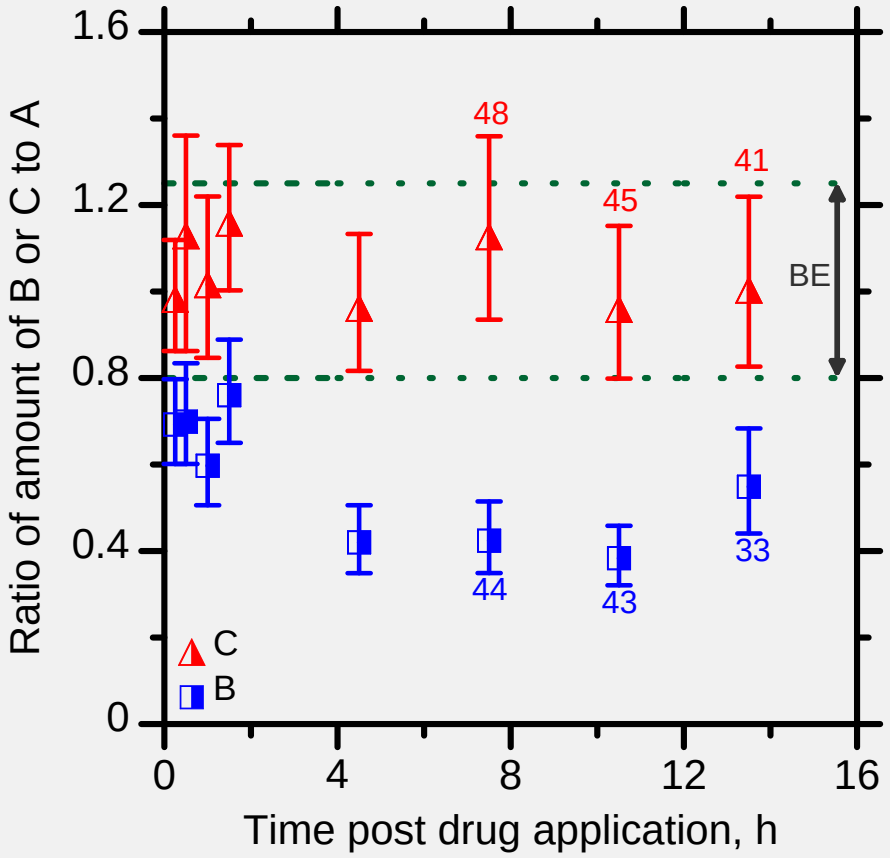

Number of detectable measurements are indicated on data points if less than 49 total

Data from Pershing; N'Dri-Stempfer *et al.*, Pharm Res, 2008

- BE determinations at individual time points are the same as from AUC or A_{max}
- Larger confidence intervals at individual time points
- Reduce by decreasing experimental variability and duplicating determinations

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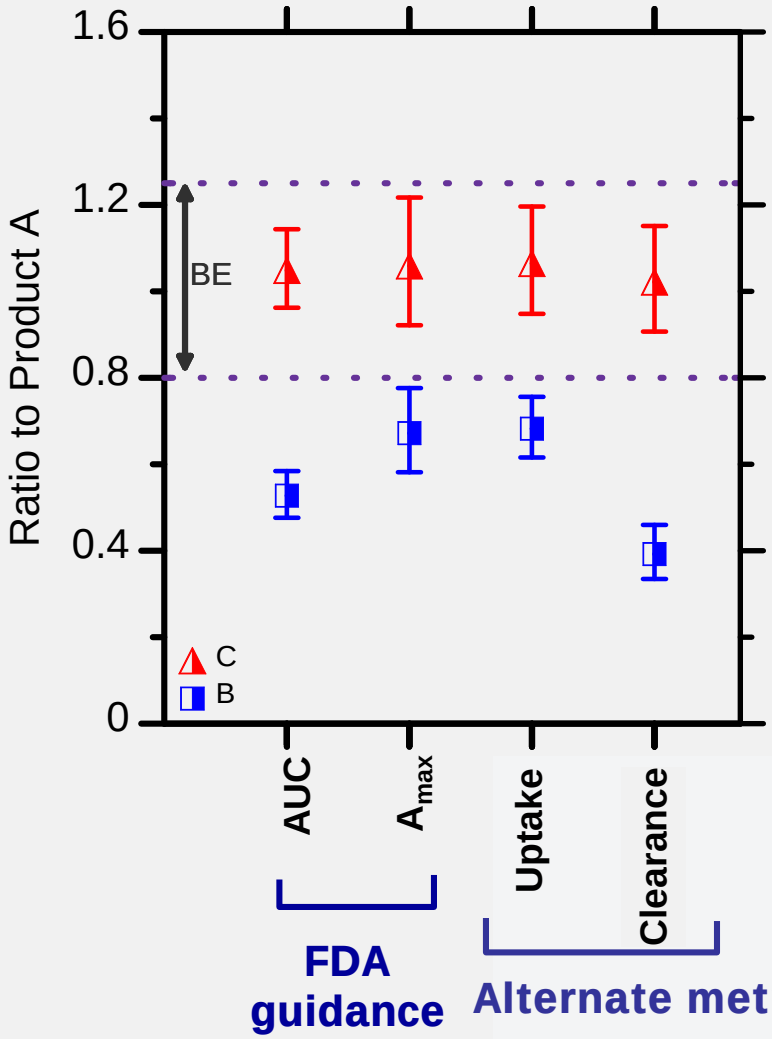

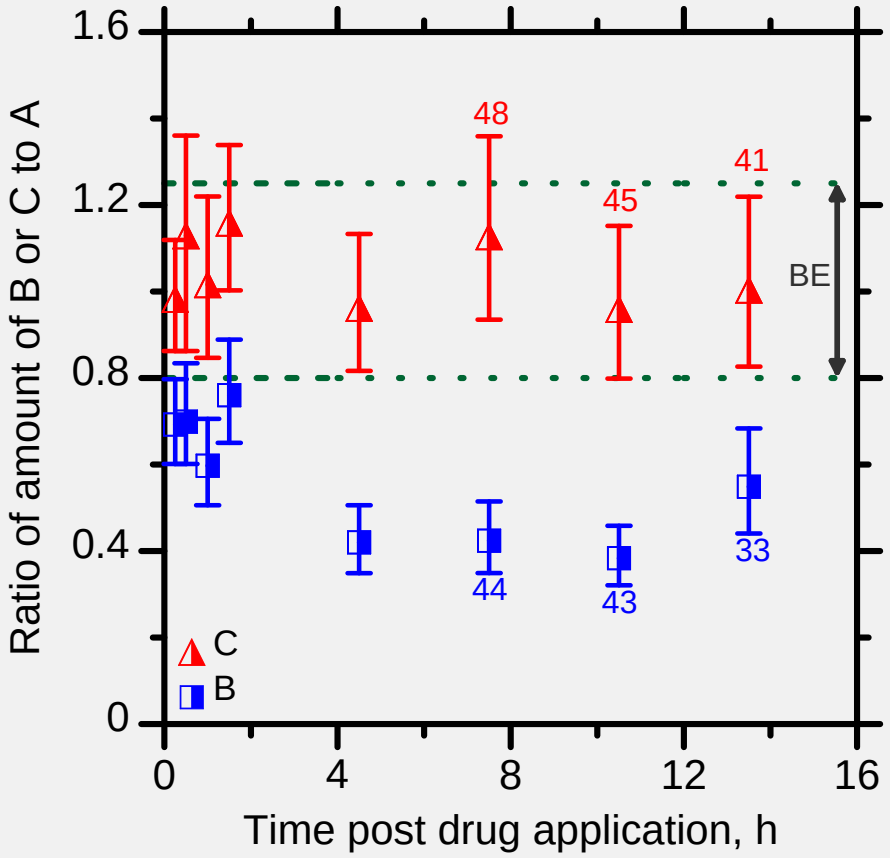

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Improved Protocol Developed for FDA

- Stripped area < drug application area (control both areas)
- 1 uptake time & 1 clearance time
  - Duplicate determinations at each time
  - 4 treatment sites / product

Simplify the method

Reduce lab-to-lab (operator) differences

- Reliably remove unabsorbed drug (isopropyl alcohol wipes)
- Reduced sample variability by improved drug collection
  - Determine ~*all* drug in SC by removing nearly all of the SC
    - Remove SC until TEWL > 8 x (TEWL before stripping)
    - At least 12 tape strips, but not more than 30 tape strips
  - Total drug amount = Drug from all tapes (no tapes discarded)

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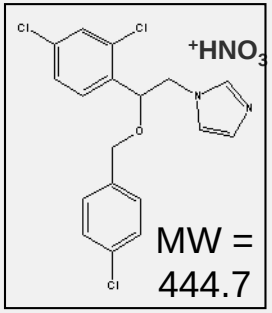
  - Decrease lab-to-lab differences (reduce sensitivity to different operators)
  - FDA → Concerned about assessing BE when target is not the SC Restricted study to antifungal drugs that target the SC

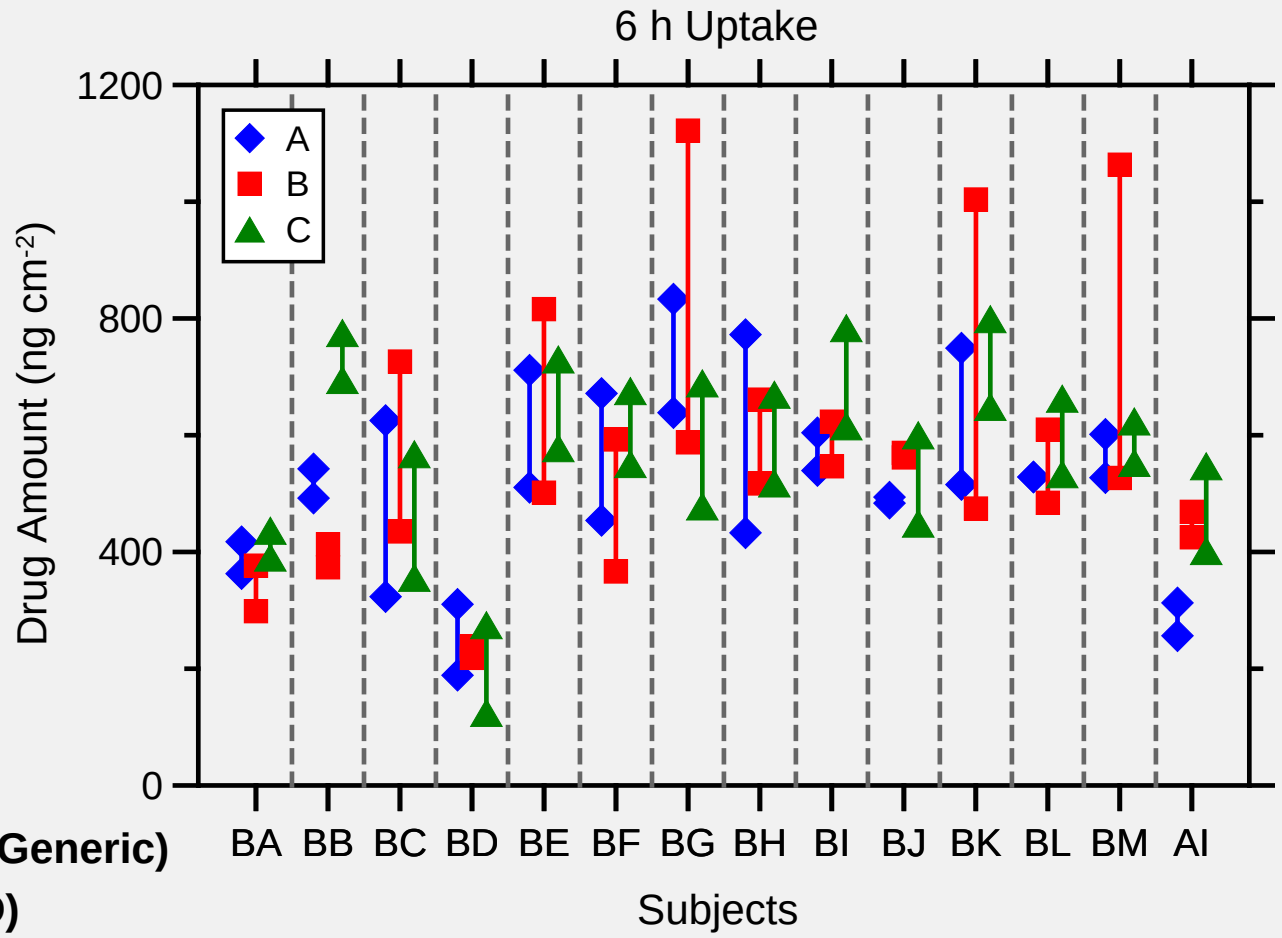
Demonstrating the Improved Protocol

- Econazole nitrate 1% cream
  - Antifungal – SC is target site
- Compare 2 generic products to RLD
  - Both products Q1 and Q2 equivalent
- 6 h uptake time & 17 h clearance time
  - Chosen based on pilot study results, and
  - Convenience for subjects and operator



Econazole *UPTAKE* into SC

Econazole nitrate from 3 bioequivalent formulations measured in duplicate (n=14)



A = Clay-Park (Generic)

B = Ortho (RLD)

C = Taro (Generic)

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Duplication of measurements improved results

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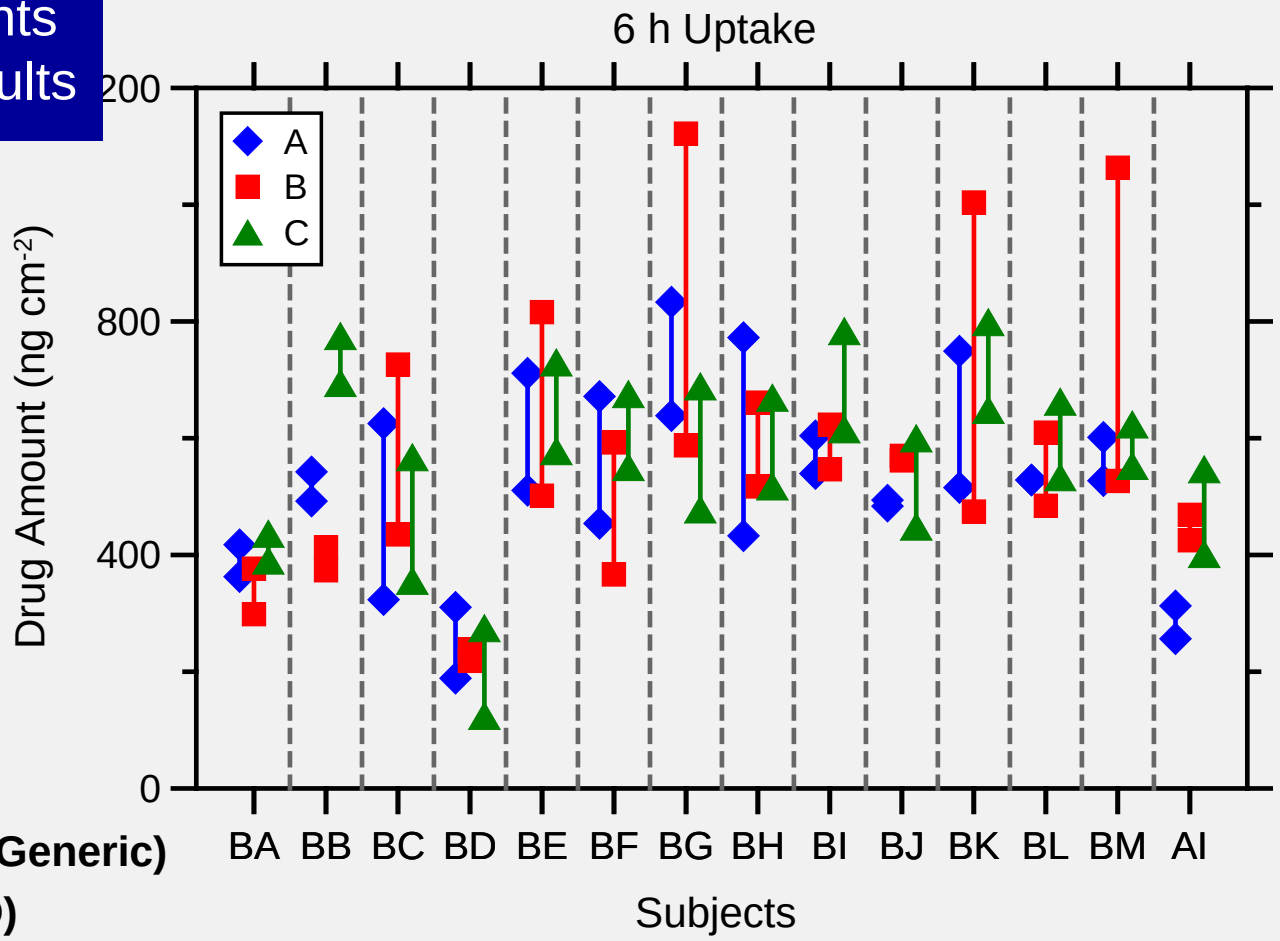

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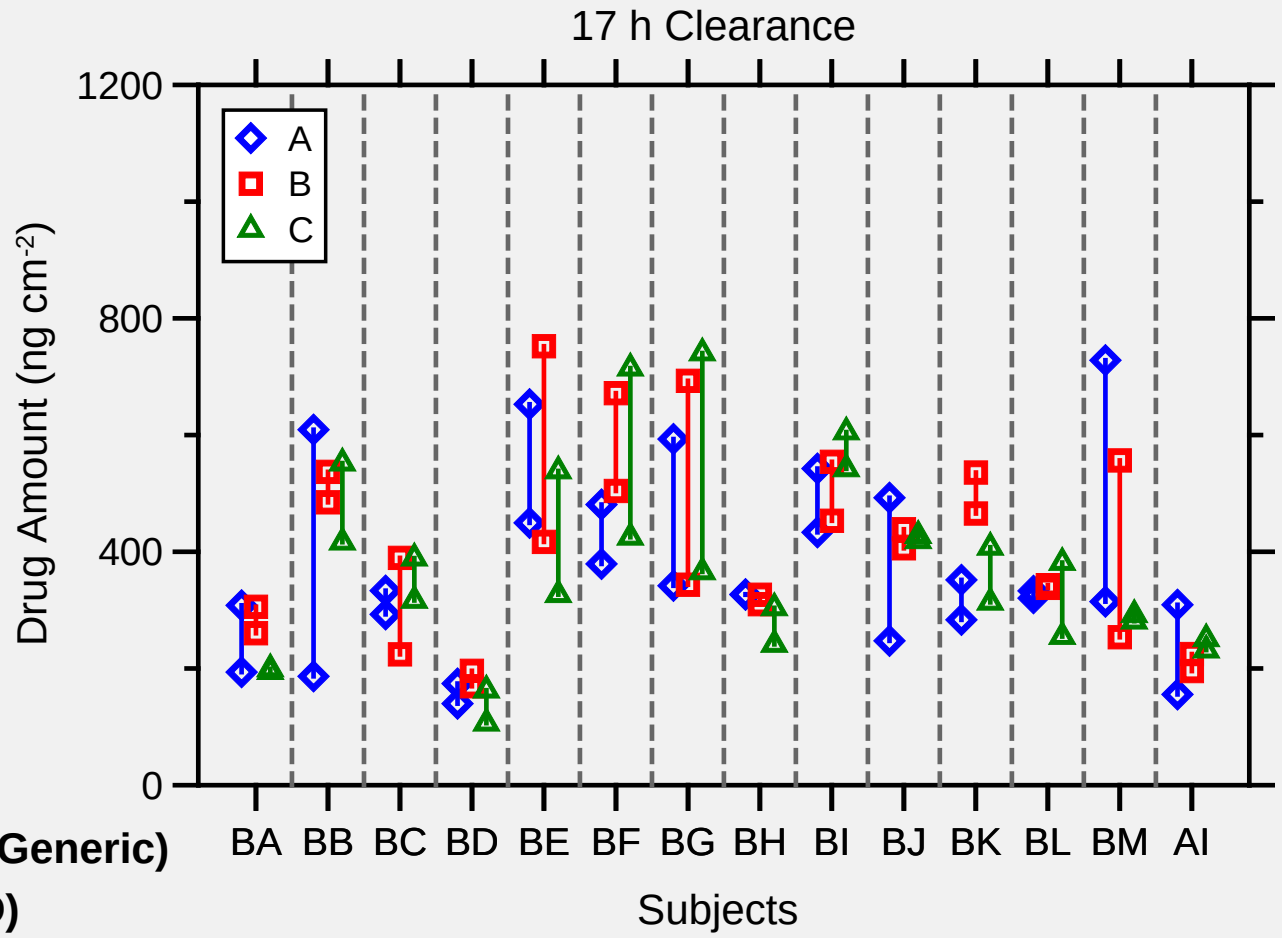

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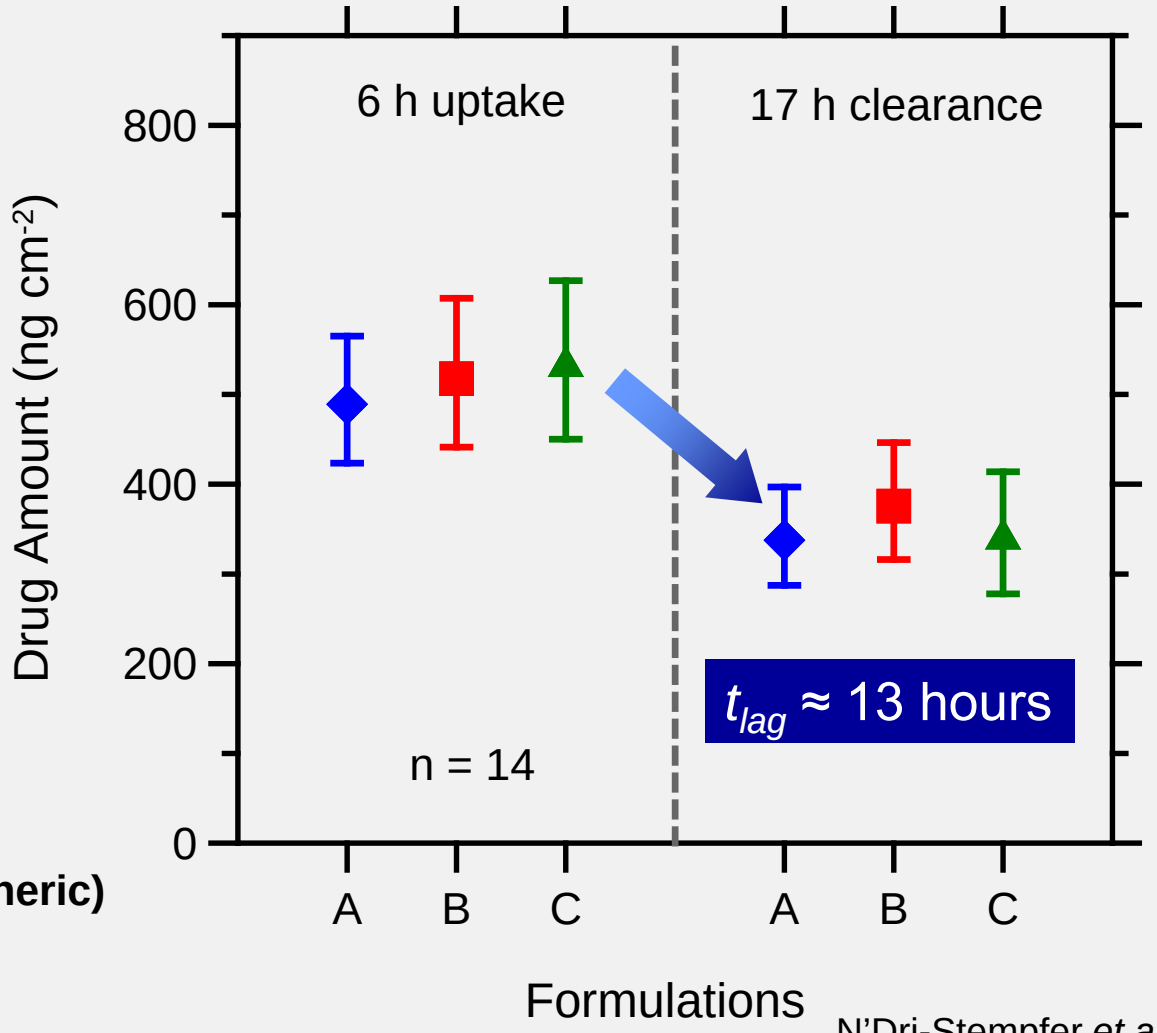
B = Ortho (RLD)

C = Taro (Generic)

N'Dri-Stempfer *et al*., Pharm Res, 2009

Econazole in SC: *Average drug amounts*



A = Clay-Park (Generic)
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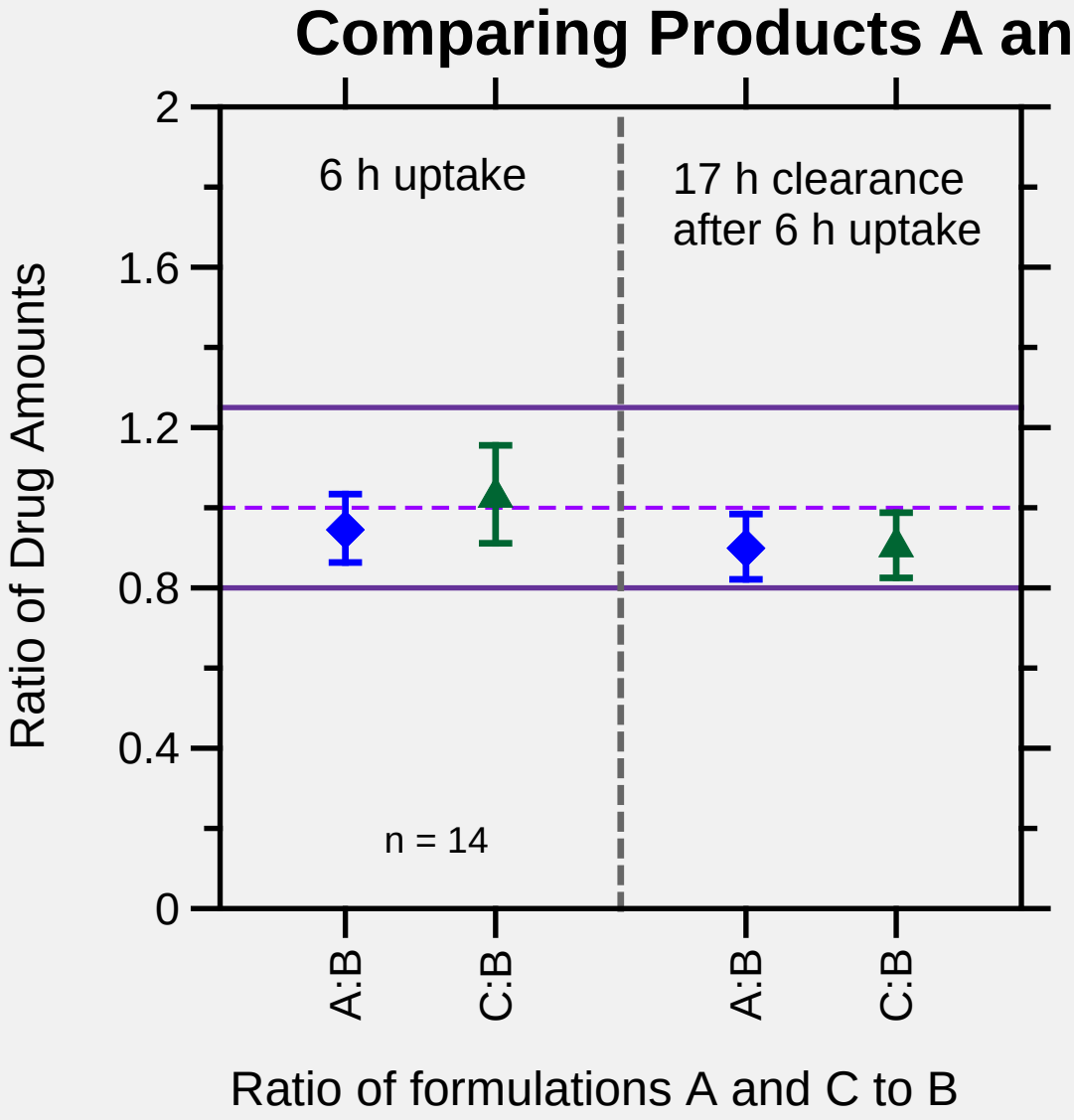
N'Dri-Stempfer *et al.*, Pharm Res, 2009

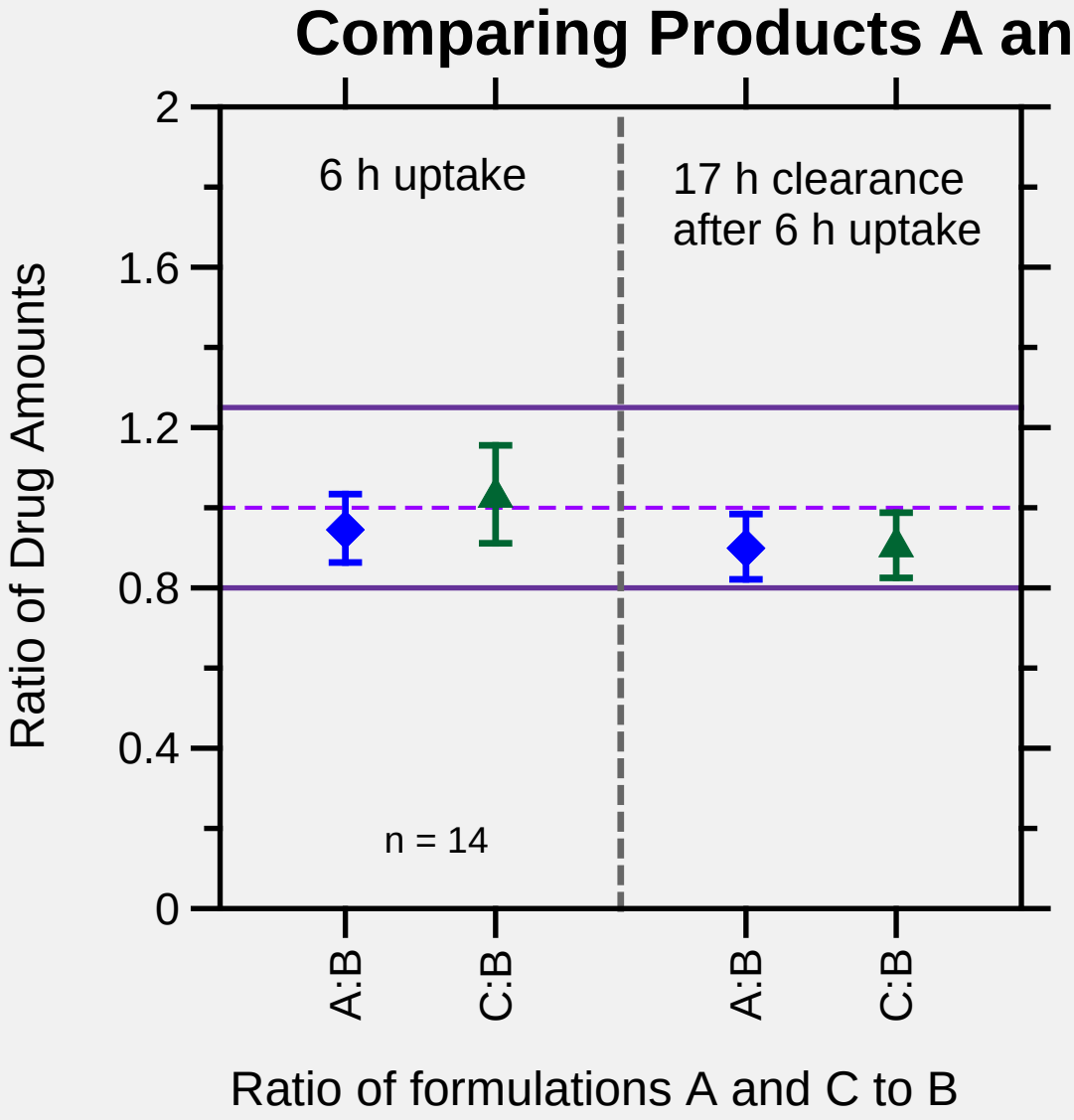
Econazole in SC: *BE assessment*



- Both A and C were conclusively BE with B after uptake and clearance, evaluated separately.

N'Dri-Stempfer *et al*., Pharm Res, 2009

Econazole in SC: *BE assessment*



- Both A and C were conclusively BE with B after uptake and clearance, evaluated separately.
- Only **168 sites** (3 products in 14 subjects with replicates for uptake & clearance = 3 x 14 x 2 x 2)
- Compare with **1176 sites** in tretinoin gel study (3 products in 49 subjects with 8 sites/product = 3 x 49 x 8)

N'Dri-Stempfer *et al*., Pharm Res, 2009

Skin Stripping: *Brief history*

- The Beginning? Rougier et al. (1983)
- 1998 – FDA guidance for Dermatopharmacokinetic (DPK) bioequivalence (BE) assessment of topically applied drugs
- 2002 – FDA guidance for DPK withdrawn
- 2003 – FDA sponsored research to improve the skin stripping method for BE assessment
  - Simplify the method (fewer analyses and decreased variability)
  - Decrease lab-to-lab differences (reduce sensitivity to different operators)
  - FDA → Concerned about assessing BE when target is not the SC

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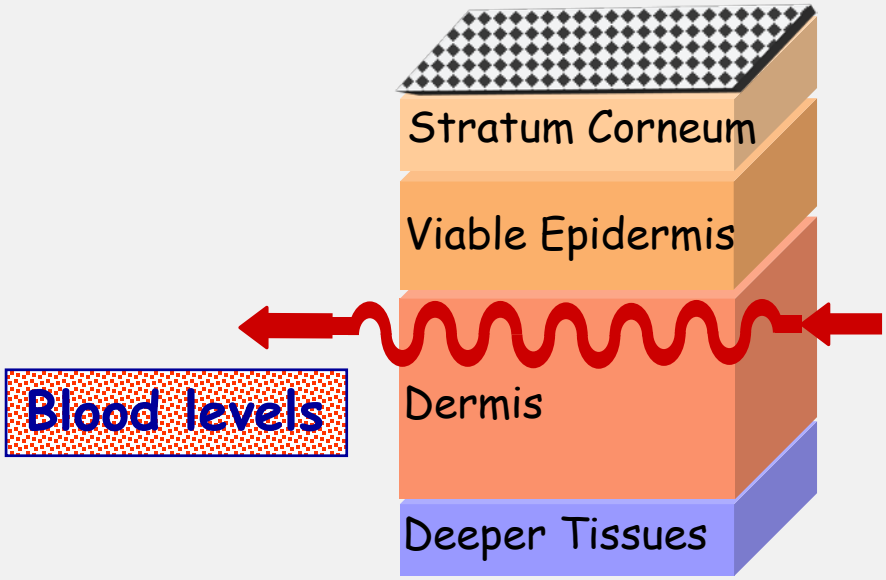
- 2013 – FDA sponsored research to assess *in vitro – in vivo* correlation

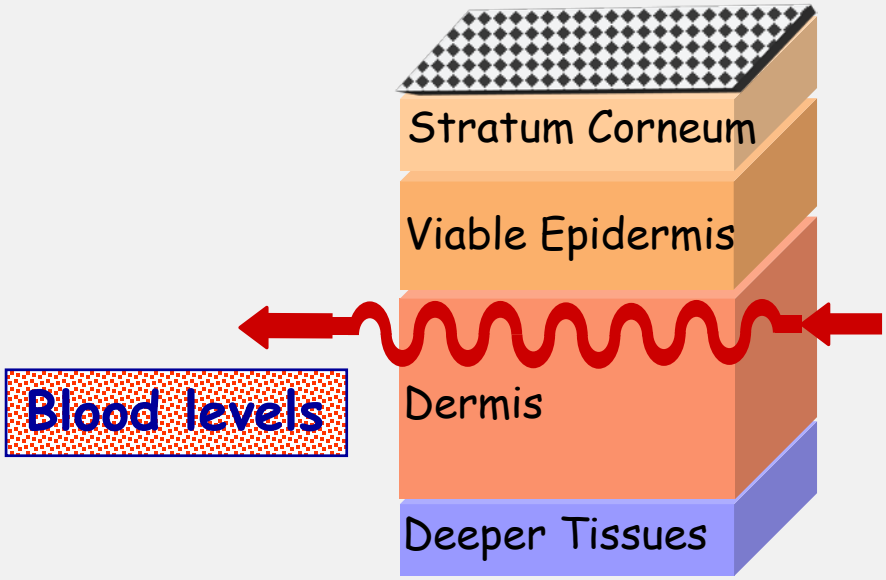
Relevance of Drug in SC to Other Skin Layers

- For target to dermis or deeper tissues, blood levels are appropriate (if large enough to measure)



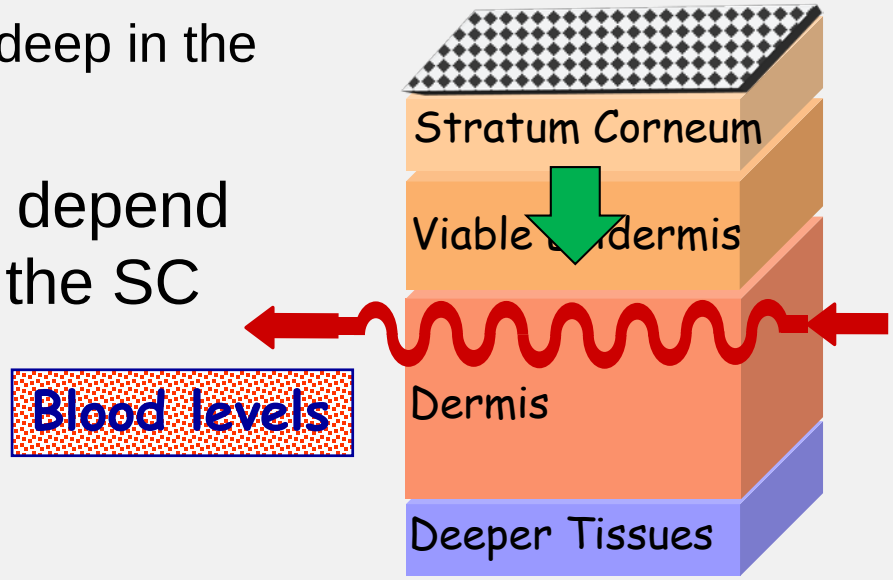
Relevance of Drug in SC to Other Skin Layers

- For target to dermis or deeper tissues, blood levels are appropriate (if large enough to measure)
- Drug levels in the SC
  - Will be a good measure of extent (how much drug is delivered), but
  - Might or might not be correlated with levels in non-SC layers



Relevance of Drug in SC to Other Skin Layers

- Drug levels in the SC might not be correlated to drug levels in the non-SC layers if:
  - Topical products include enhancers that evaporate or permeate faster than the drug
    - Enhancer pushes excess drug into the SC
    - Drug “stranded” in SC when the enhancer is depleted
  - Non-absorbed drug is trapped deep in the microfurrows of the SC
- Drug levels in non-SC layers depend on rate of drug delivery from the SC
- **Drug delivery rate from SC is an appropriate surrogate for drug levels in non-SC layers**



Determine Delivery Rate from SC Drug Levels

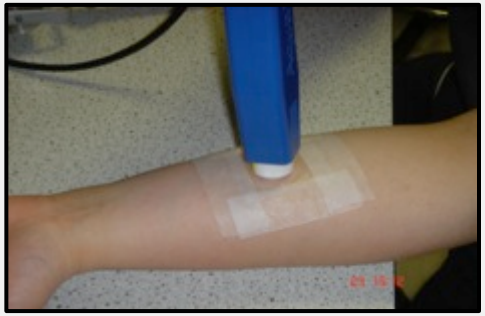
Method 1: Measure drug concentration profile in the SC before steady state to determine:

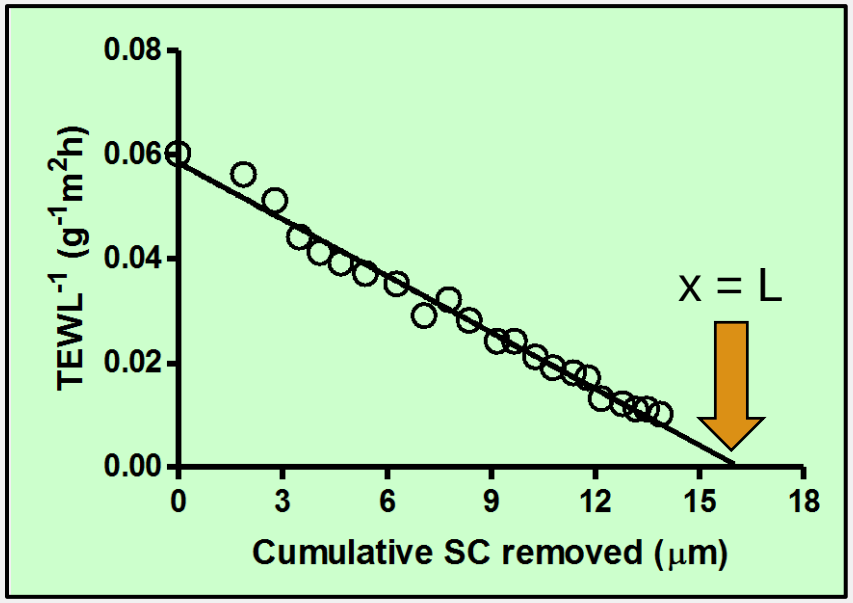
- Partitioning to SC
- Diffusion through SC
- Permeation through SC (partitioning $\times$ diffusion) **= RATE**

Drug Concentration Profile in SC

Determine:

- Amount of SC on tapes
- Combine with TEWL to determine SC thickness

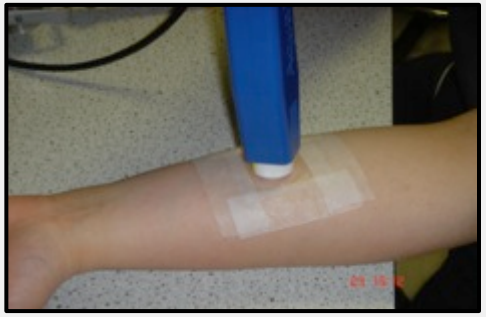


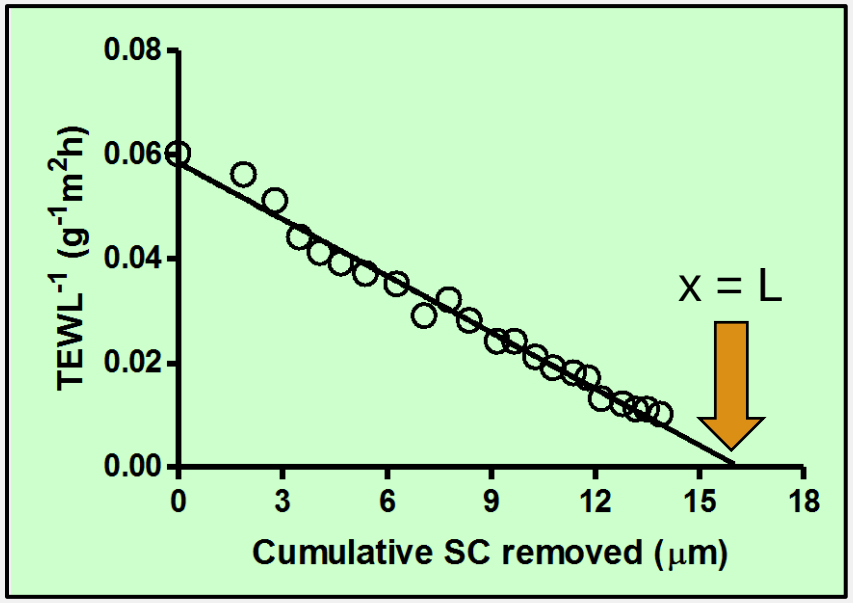


Drug Concentration Profile in SC

Determine:

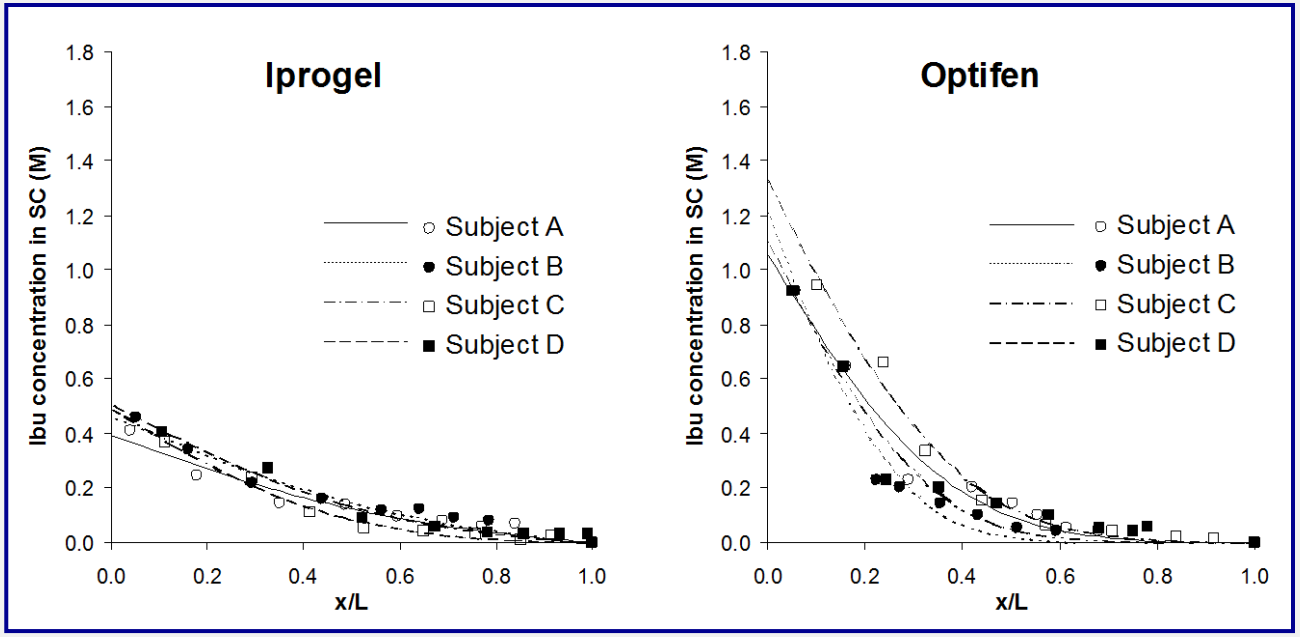
- Amount of SC on tapes
- Combine with TEWL to determine SC thickness
- Drug levels on tapes





Drug Concentration Profile in SC

5% ibuprofen gel applied 0.5 h



$$C = K C_{vehicle}\left[1 - \frac{x}{L} - \frac{2}{\pi}\sum_{n=1}^{\infty}\frac{\sin(n\pi\, x/L)}{n}\exp\left(-\frac{D}{L^2}n^2\pi^2 t\right)\right]$$

Herkenne et al. JID, 127:135-142 (2007)

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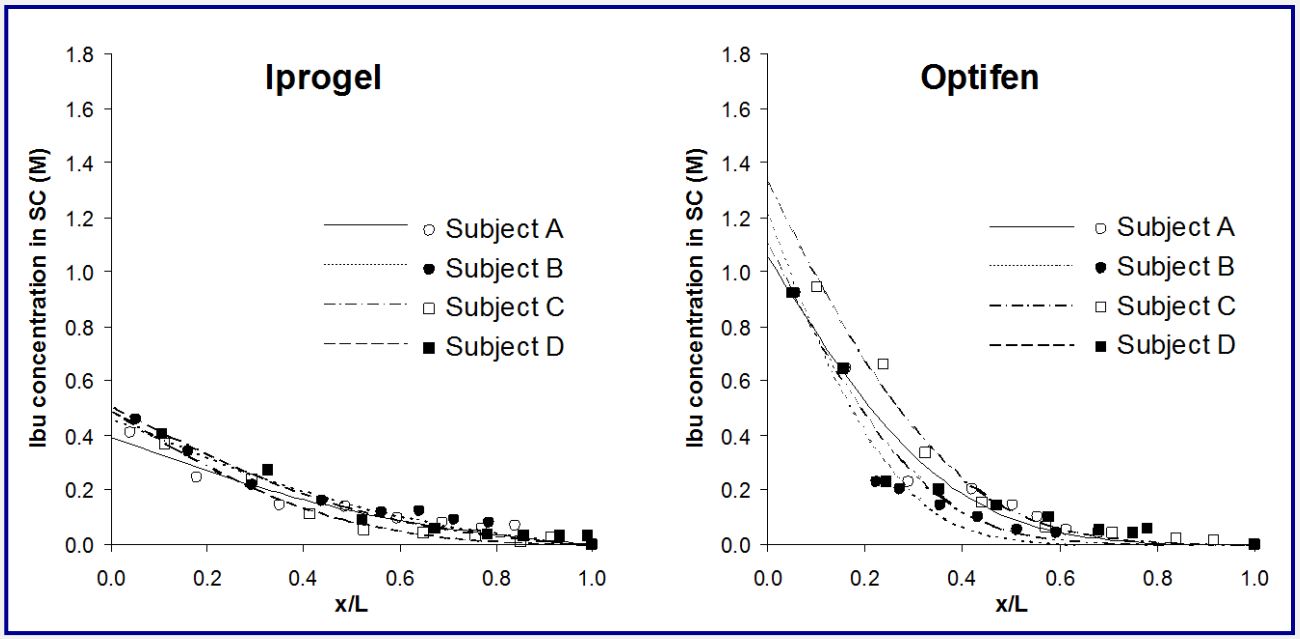

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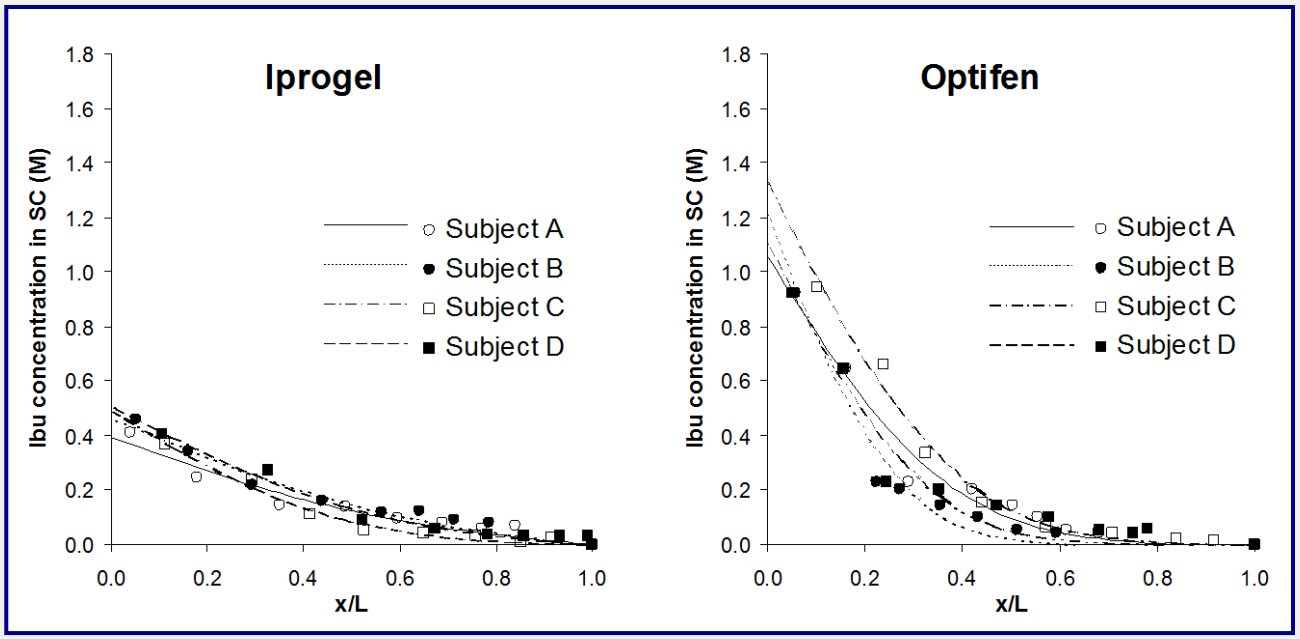


Parameter	Iprogel	Optifen
K	1.92 ± 0.21	4.80 ± 0.47
D/L^2 [h^{-1}]	0.211 ± 0.055	0.072 ± 0.022
$k_p \times 10^3$ [cm h^{-1}]	0.51 ± 0.14	0.43 ± 0.13

Herkenne et al. JID, 127:135-142 (2007)

Determine Delivery Rate from SC Drug Levels

Method 1: Measure drug concentration profile in the SC before steady state to determine:

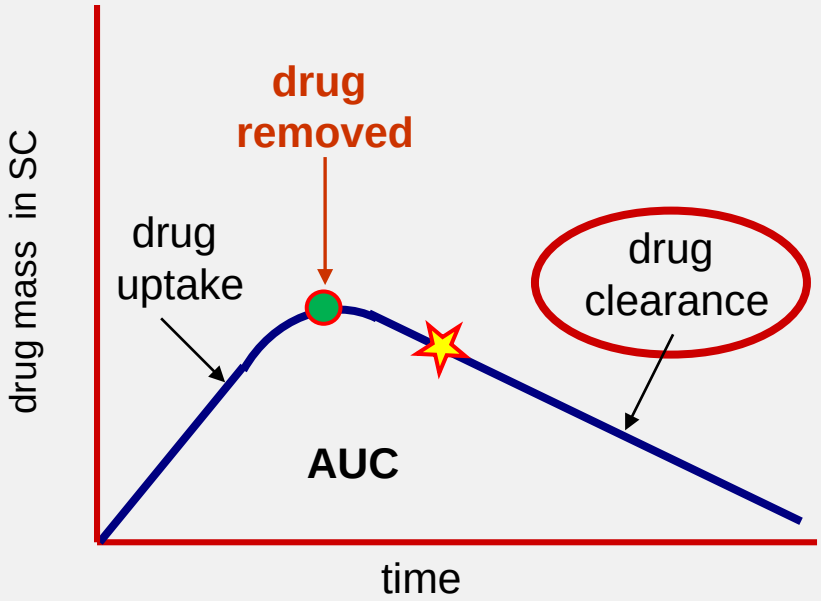
- Partitioning to SC
- Diffusion through SC
- Permeation through SC (partitioning × diffusion) **= RATE**
- Limitations
  - Complex: For each tape (or few tape strips) measure drug levels, amount of SC, and TEWL
  - Limited to short time (unsteady-state) exposures
  - Sensitive to non-absorbed drug left in “microfurrows” after cleaning

Determine Delivery Rate from SC Drug Levels

Method 2: Measure clearance rate from drug levels in SC after drug is removed

- Measure mass of drug in SC after clearance (✸)
- Compare to mass of drug in SC after uptake ends (●)

- Calculate the average flux from the SC



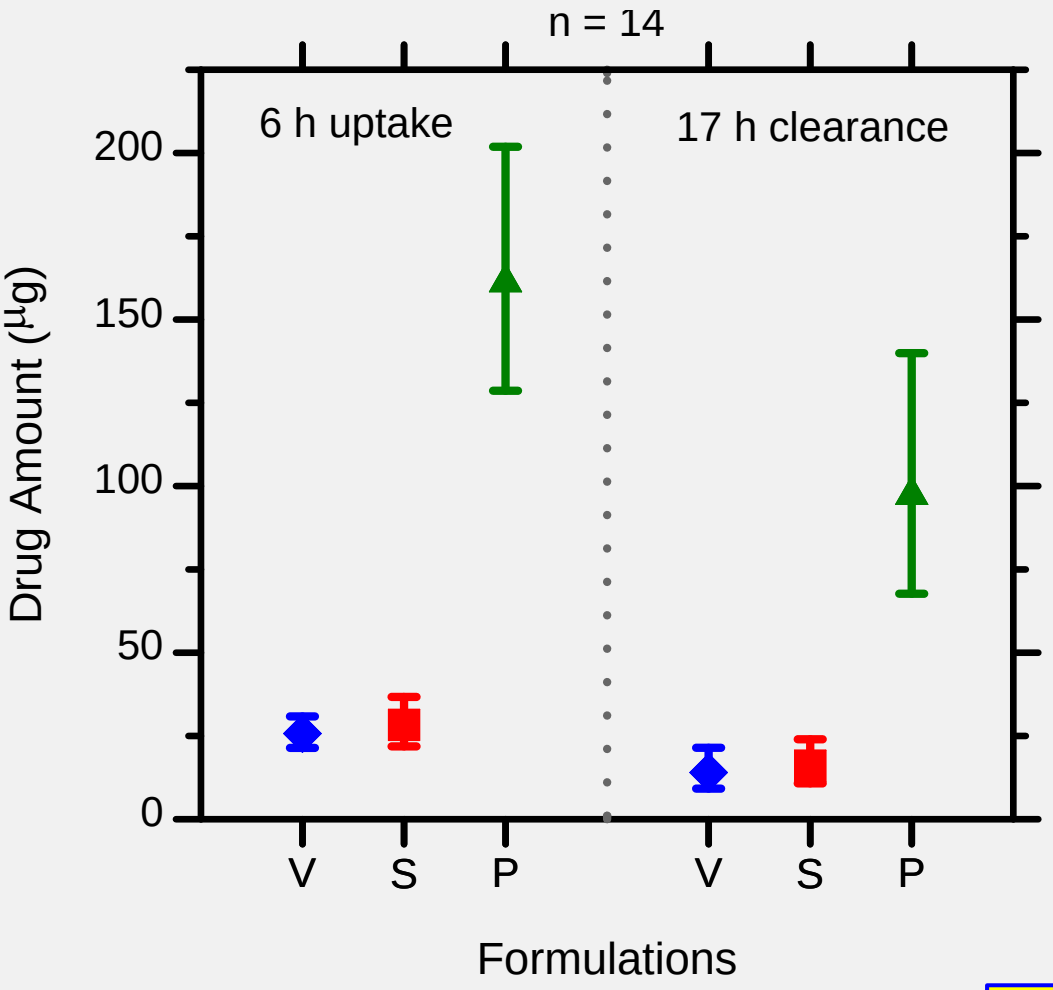
$$\text{Average Flux} = \frac{\left(M_{drug,t_1} - M_{drug,t_2}\right)/A}{t_2 - t_1}$$

Average Flux from Drug Levels in SC (I)

DICLOFENAC

- Compare 3 products
  - 2% solution (Pennsaid) – 10 mg/cm^2 (contains DMSO)
  - 3% gel (Solaraze) – 20 mg/cm^2
  - 1% gel (Voltaren) – 10 mg/cm^2
- 17 h clearance time after 6 h uptake
- 14 subjects

Diclofenac: *Average drug amounts in SC*

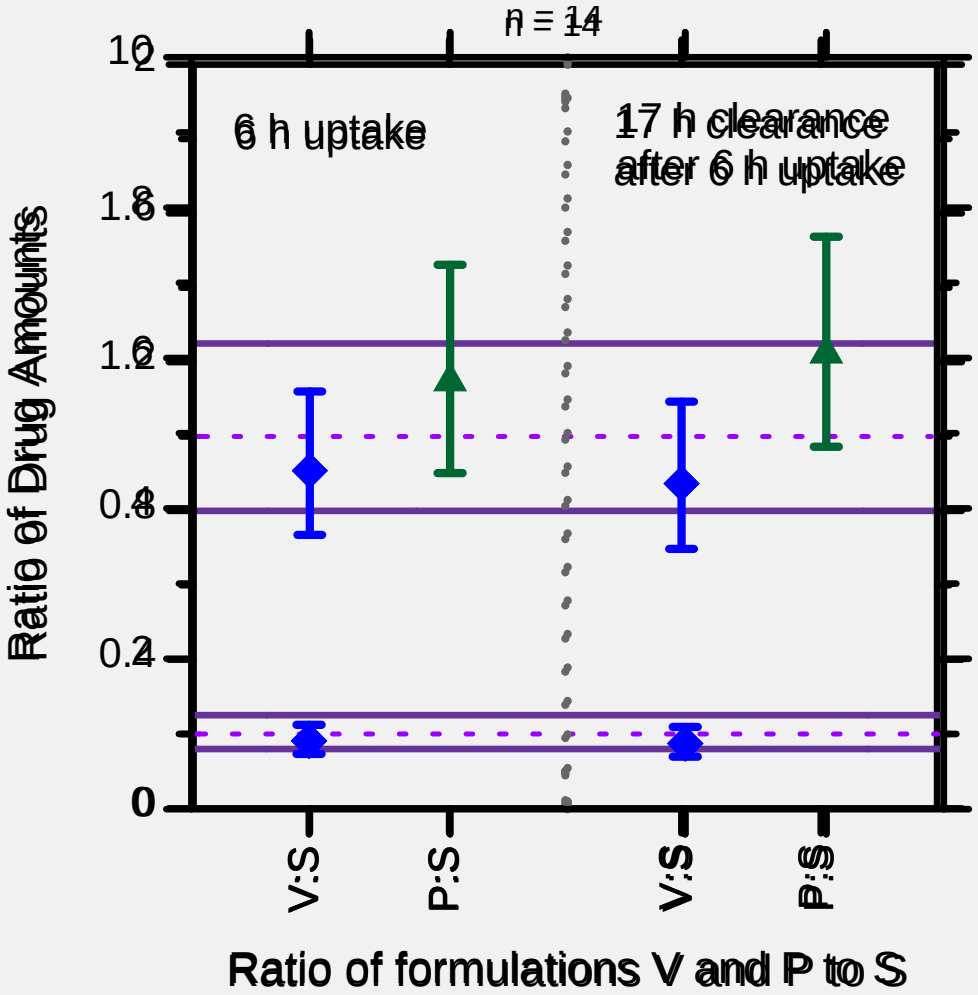


V = Voltaren (1%)
S = Solaraze (3%)
P = Penssaid (2%)

Error bars, 90% CI of the log mean

Diclofenac: *BE ratio of drug amounts in SC*

Comparing Products V and P to Product S

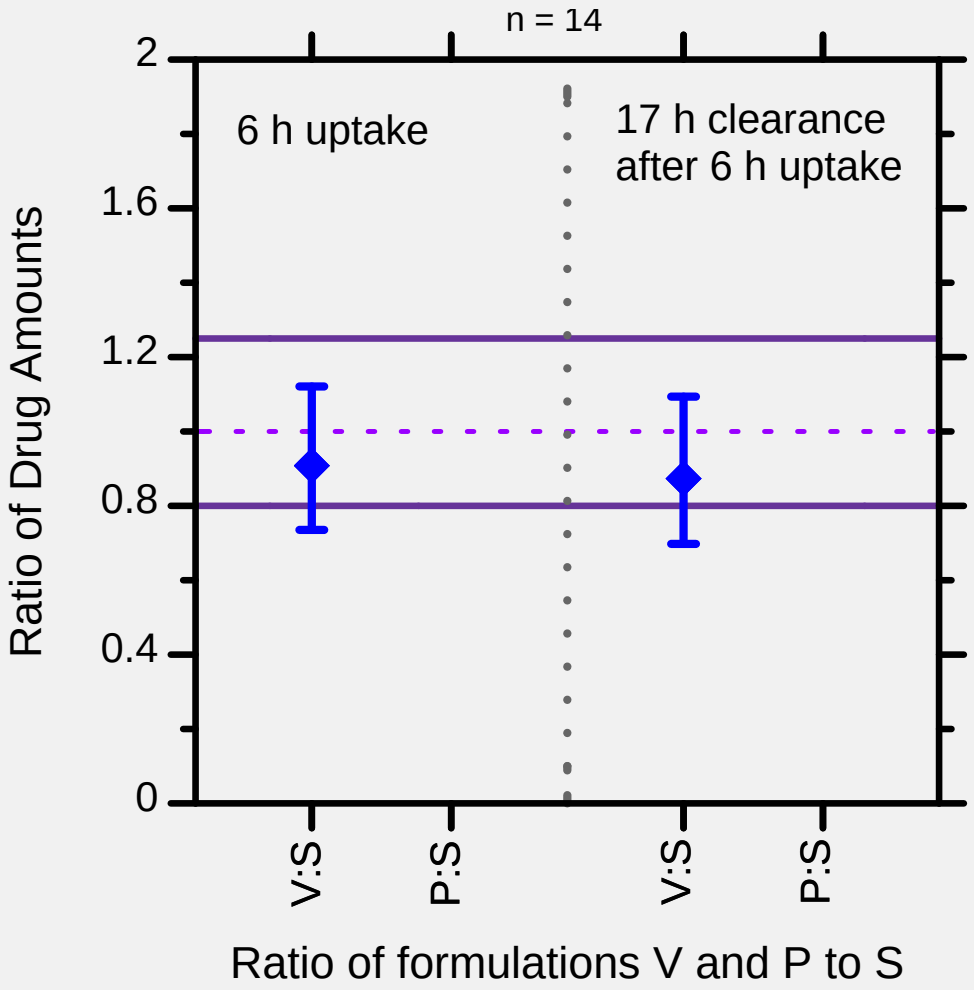


V = Voltaren (1%)
S = Solaraze (3%)
P = Penssaid (2%)

Error bars, 90% CI of the log mean
Dashed horizontal line = 1
Solid lines @ 0.8 & 1.25

Diclofenac: *BE ratio of drug amounts in SC*

Comparing Products V and P to Product S



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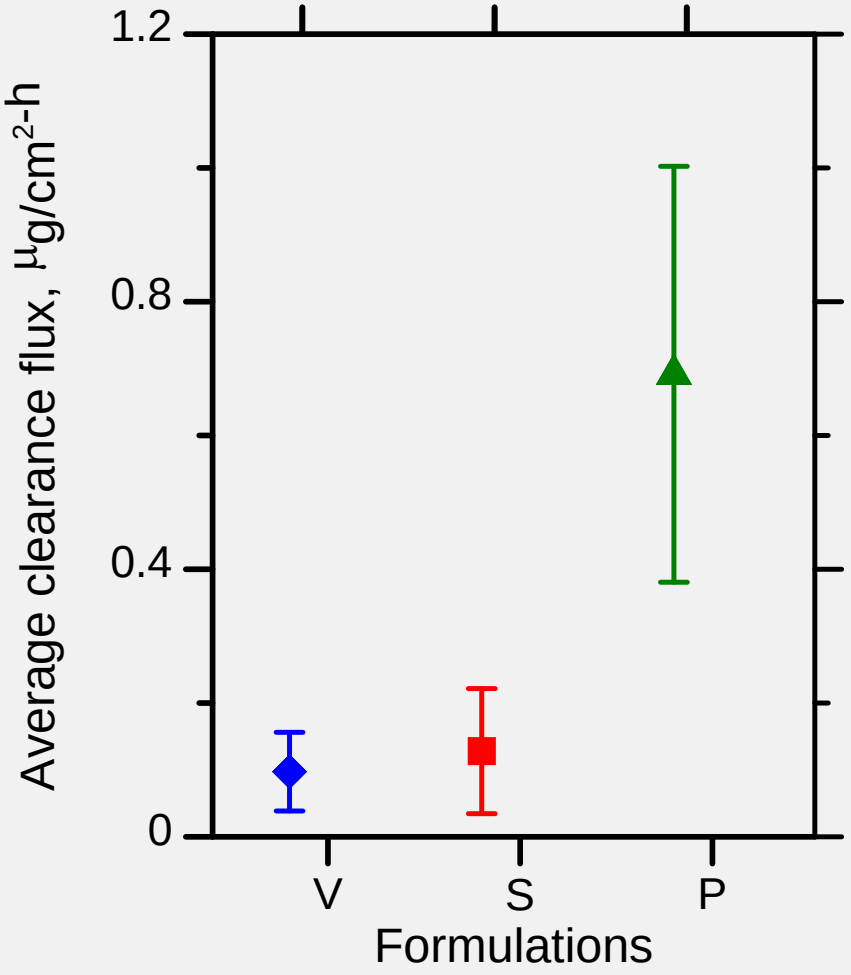
Error bars, 90% CI of the log mean
Dashed horizontal line = 1
Solid lines @ 0.8 & 1.25

Diclofenac: *Average clearance flux*



V = Voltaren (1%)
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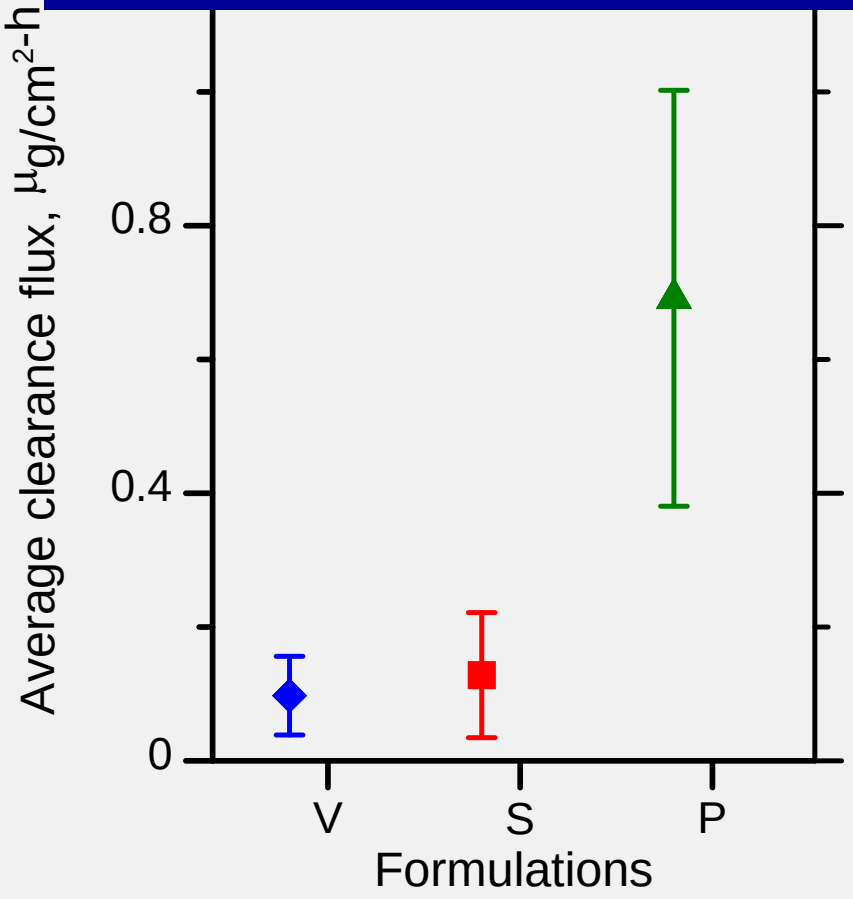
P = Penssaid (2%)

Error bars, 90% CI

Diclofenac: *Average clearance flux*

Do these estimates of average clearance represent drug delivery through SC?



V = Voltaren (1%)
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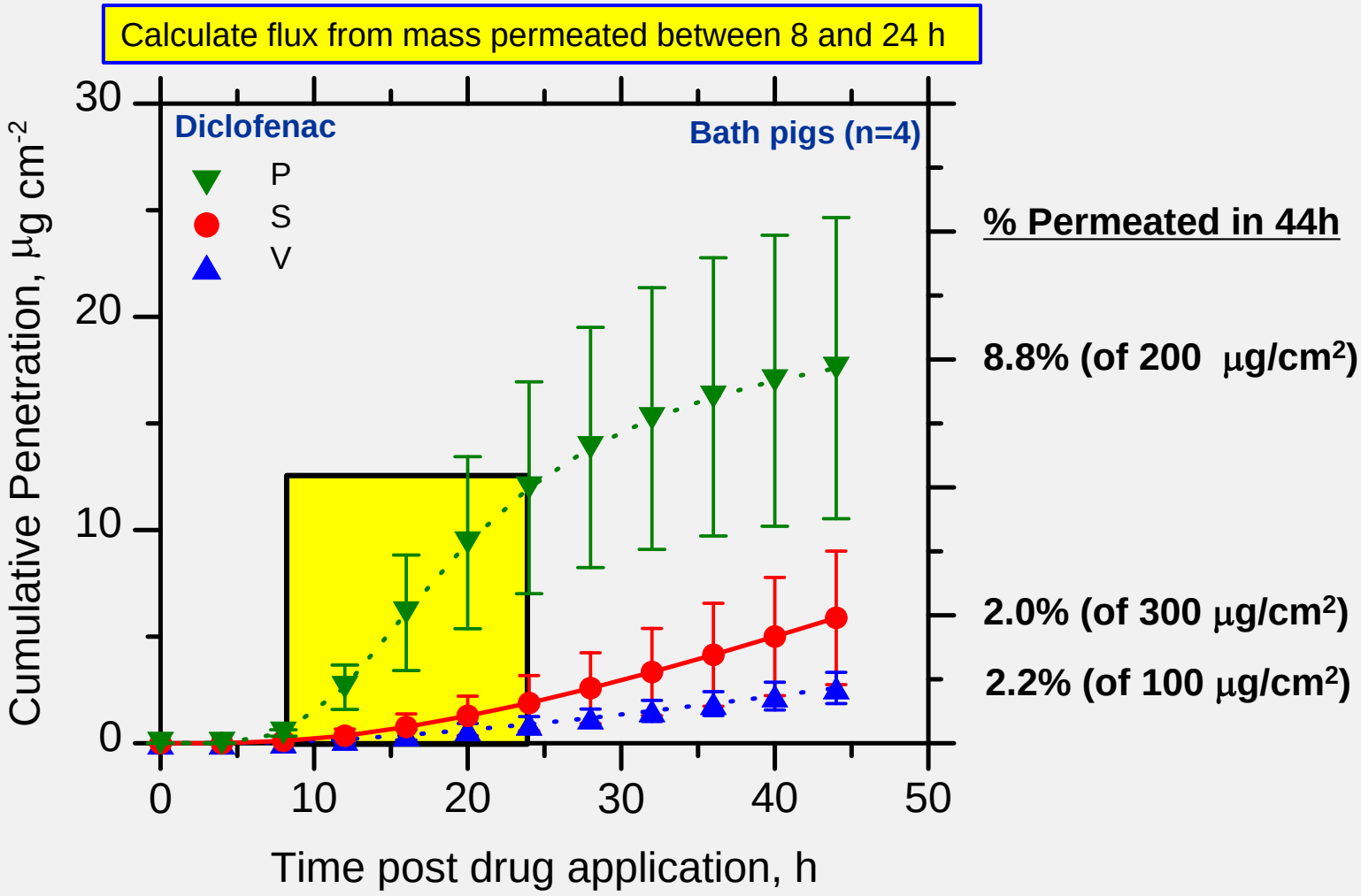
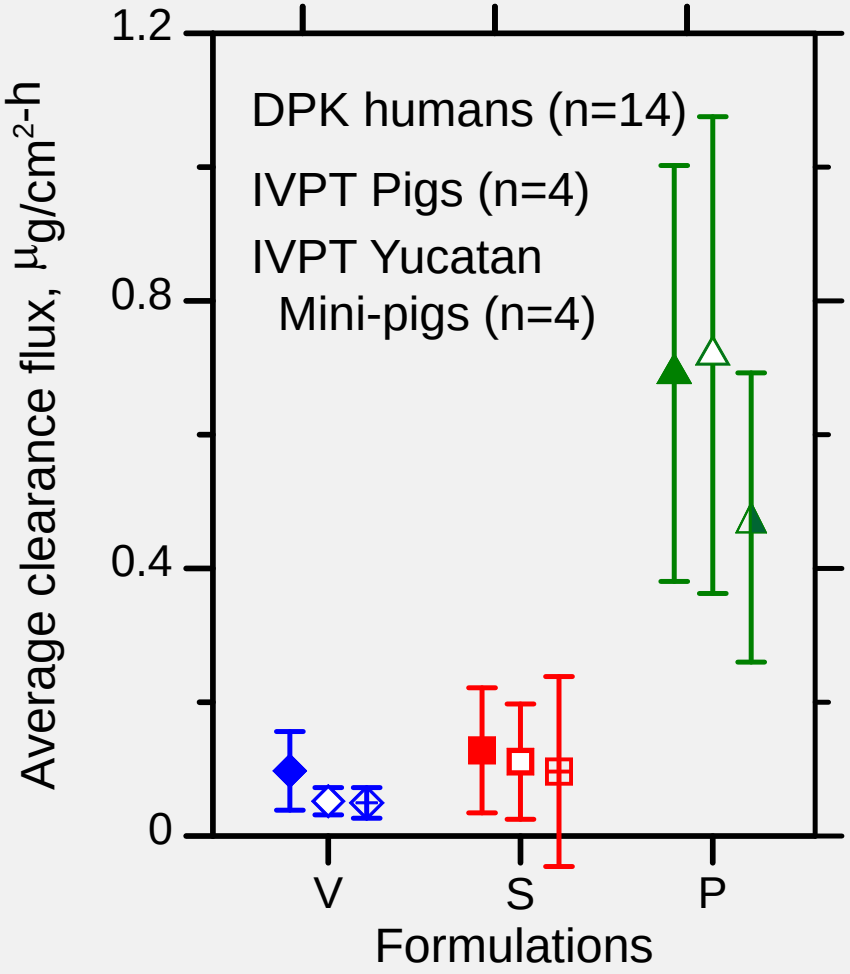
Error bars, 90% CI

Diclofenac: *In vitro permeation test results*



Diclofenac: *Average clearance flux*

DPK vs. IVPT for cumulative penetration during “clearance”



V = Voltaren (1%)

S = Solaraze (3%)

P = Penssaid (2%)

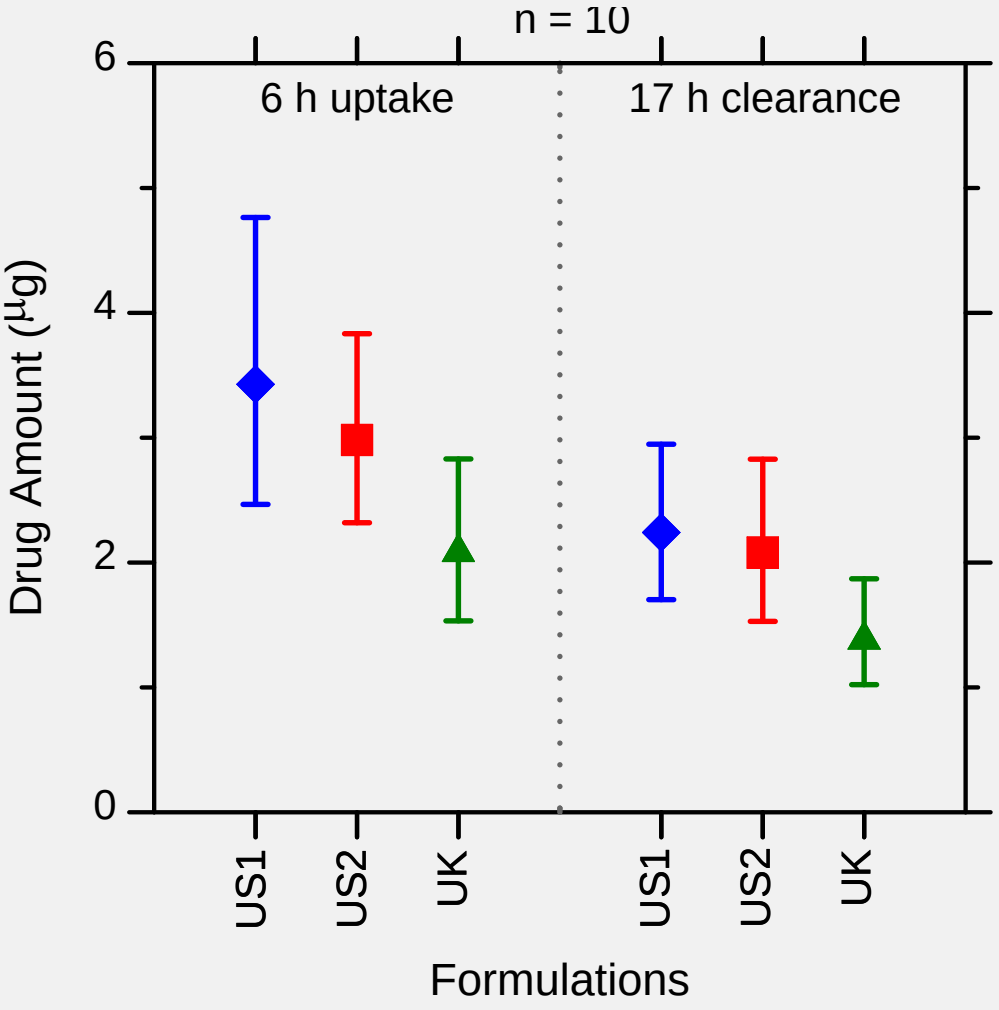
Error bars, 90% CI

Average Flux from Drug Levels in SC (II)

ACYCLOVIR

- Compare 2 products
  - US Zovirax
  - UK Zovirax
- 17 h clearance time after 6 h uptake
- 10 subjects

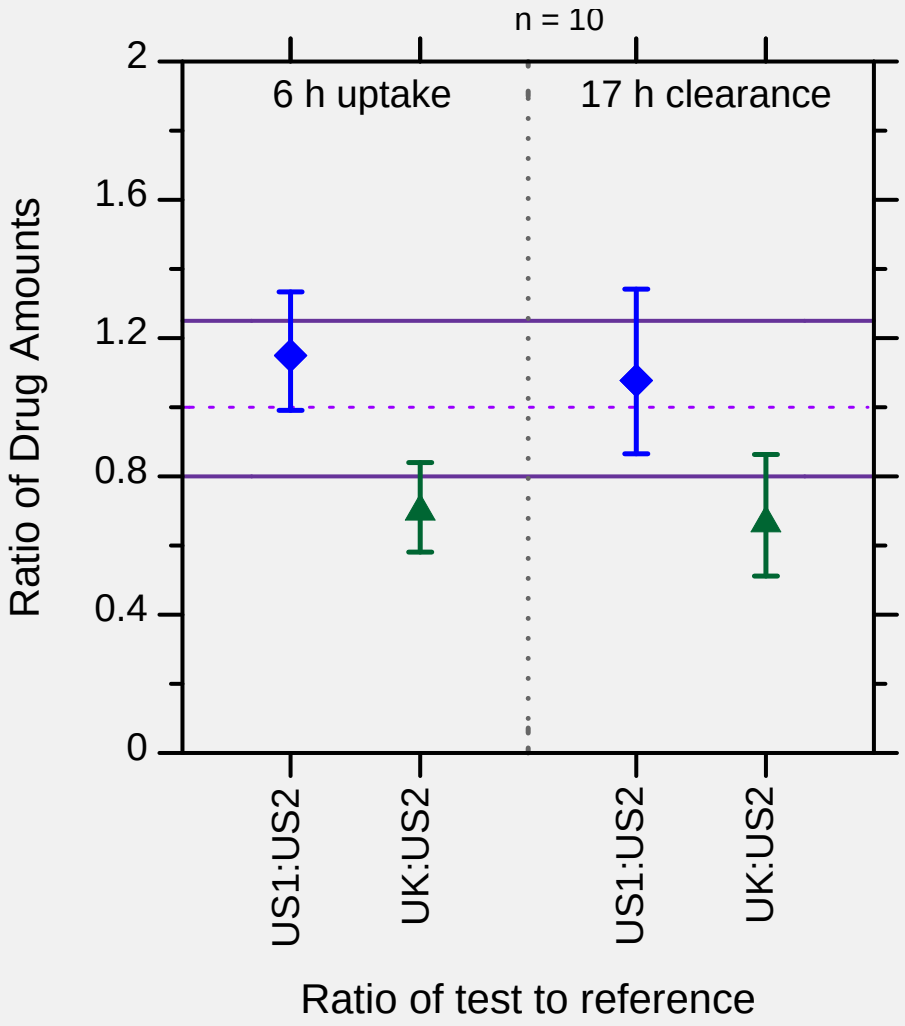
Acyclovir: *Average drug amounts in SC*



Error bars, 90% CI of the log mean

Acyclovir: *BE ratio of drug amounts in SC*

Comparing US1 and UK to US2



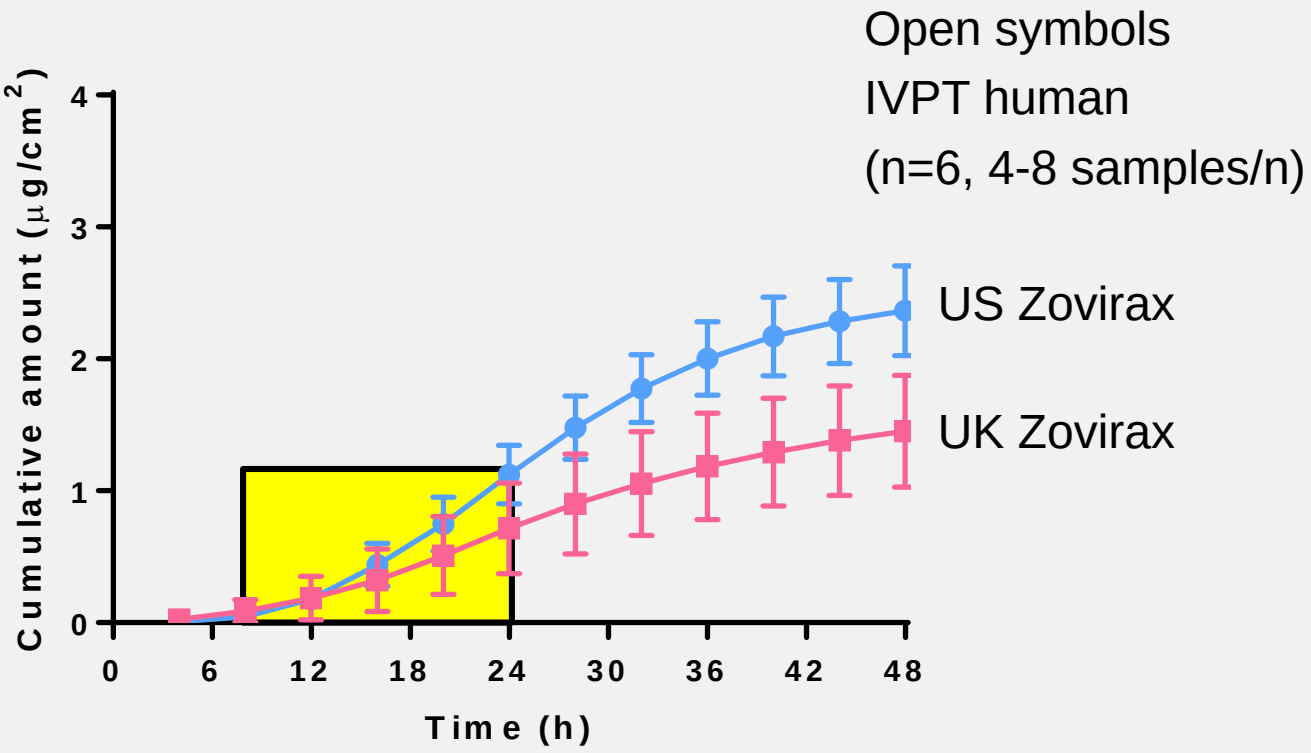
Error bars, 90% CI of the log mean

Dashed horizontal line = 1

Solid lines @ 0.8 & 1.25

Acyclovir: *In vitro permeation test results*

Calculate flux from mass permeated between 8 and 24 h



Acyclovir: *Average clearance flux*

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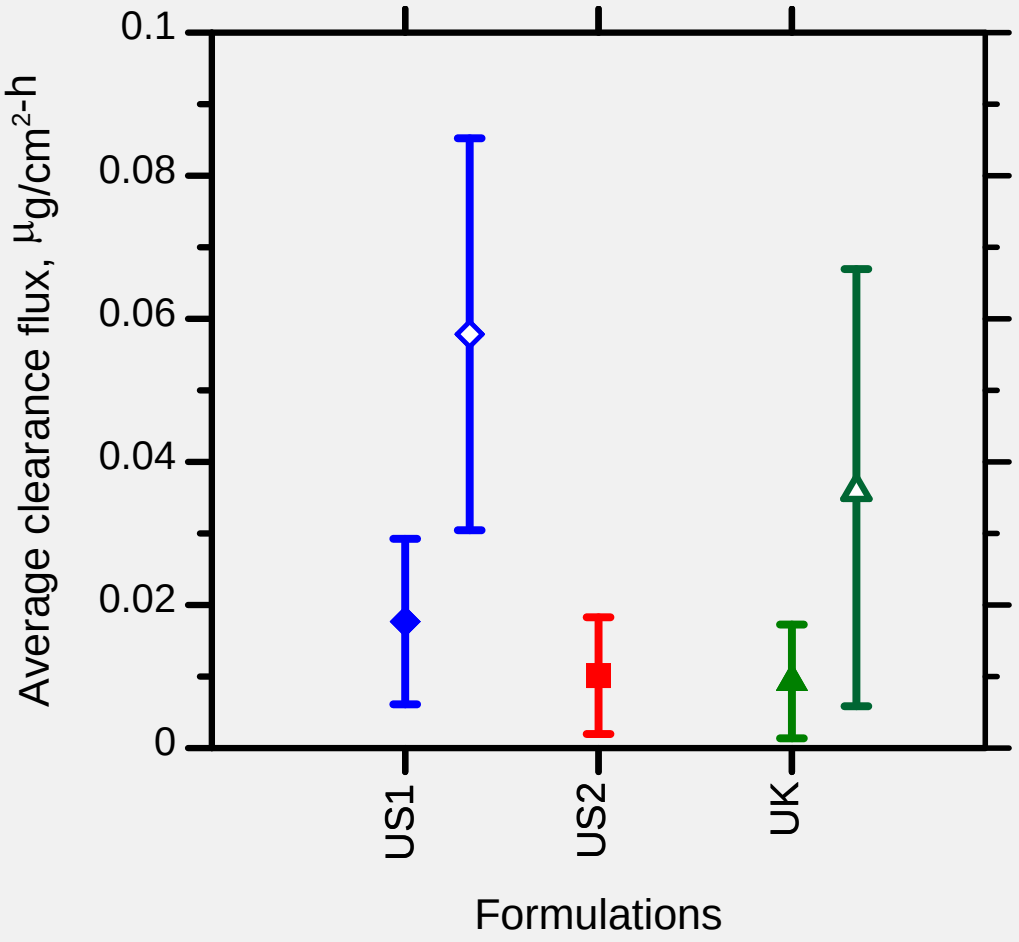

Filled symbols
DPK humans (n=14)

Open symbols
IVPT human
(n=7, 4-8 samples/n)

Skin stripping: Where are we?

- Improved skin stripping methods can reliably and efficiently assess BE of topical dermatological products
  - Pharmacokinetic (multiple time point AUC) analysis is unnecessary
  - Will FDA accept skin stripping for BE assessment?
- Skin stripping can be used to assess drug delivery rate to local target tissues other than SC
  - Results are consistent with *in vitro* permeation testing (IVPT)
  - Skin stripping can assess BE of drugs targeting non-SC tissues

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- Skin stripping can be used to assess drug delivery rate to local target tissues other than SC
  - Results are consistent with *in vitro* permeation testing (IVPT)
  - Skin stripping can assess BE of drugs targeting non-SC tissues
- Further development of method for regulatory applications
  - What metric should be assessed? BE limits (0.8-1.25, 0.75-1.33)?
  - Measurement times? How many applications? 1x or more?

Acknowledgements / Disclaimer

- US FDA funding made studies possible, in part, of econazole (award 223-04-3004), and diclofenac and acyclovir (grant 1U01FD004947-01)

- No endorsement from the FDA or US government should be inferred.*

*The views expressed in this presentation do not reflect the official policies of the US FDA or the US DHHS; nor does any mention of trade names, commercial practices, or organization imply endorsement by the US Government.

Acknowledgements

- CSM: Econazole
  - B N'Dri Stempfer, A Bowers, W Navidi
- U Bath: Diclofenac & Acylovir
  - MB Delgado-Charro, A Pensado-Lopez, WS Chiu, SF Cordery, MZ Shehab
- U Maryland Baltimore: Acyclovir
  - AL Stinchcomb, SH Shin, H Hassan, J Quarterman, D Hammell
- T Franz
- FDA: S Raney
- Volunteers who gave up skin and time

The end

Questions?