



charles river

accelerating drug development. exactly.

Evaluation of a Rapid Tritiated Water Skin Barrier Integrity Method for Use in Regulatory Toxicology Testing *in vitro*

Dr Clive Roper

Manager, Percutaneous Absorption

In vitro Sciences, Charles River, Edinburgh, UK

Introduction

- OECD Test Guideline 428 recommends the use of a barrier integrity assessment for *in vitro* percutaneous penetration studies used for risk assessment purposes
 - Pre-study Integrity Evaluation
 - Electrical Resistance
 - TEWL
 - Tritiated water
 - Concurrent Integrity Evaluation
 - Post-study Integrity Evaluation

Published Rejection Criteria for k_p

Reference	Rejection Criterion (x 10 ⁻³ cm/h)
Bronaugh <i>et al</i> (1986)	2.5
Scott <i>et al</i> (1987)	1.5
Van de Sandt <i>et al</i> (2000)	2.0
Buist <i>et al</i> (2005)	4.0
Meidan & Roper (2008)	4.5*

Why These Differences?

- Low rejection values were the median value obtained in the data sets
 - These reject intact skin with a poor barrier function
 - Not acceptable for a risk assessment
- Higher values include skin which is intact but with a poor barrier function and were calculated as up to 90% confidence interval

Why Do We Need A Barrier Integrity Test?

- To demonstrate if the skin is
 - intact or
 - has become damaged at any point

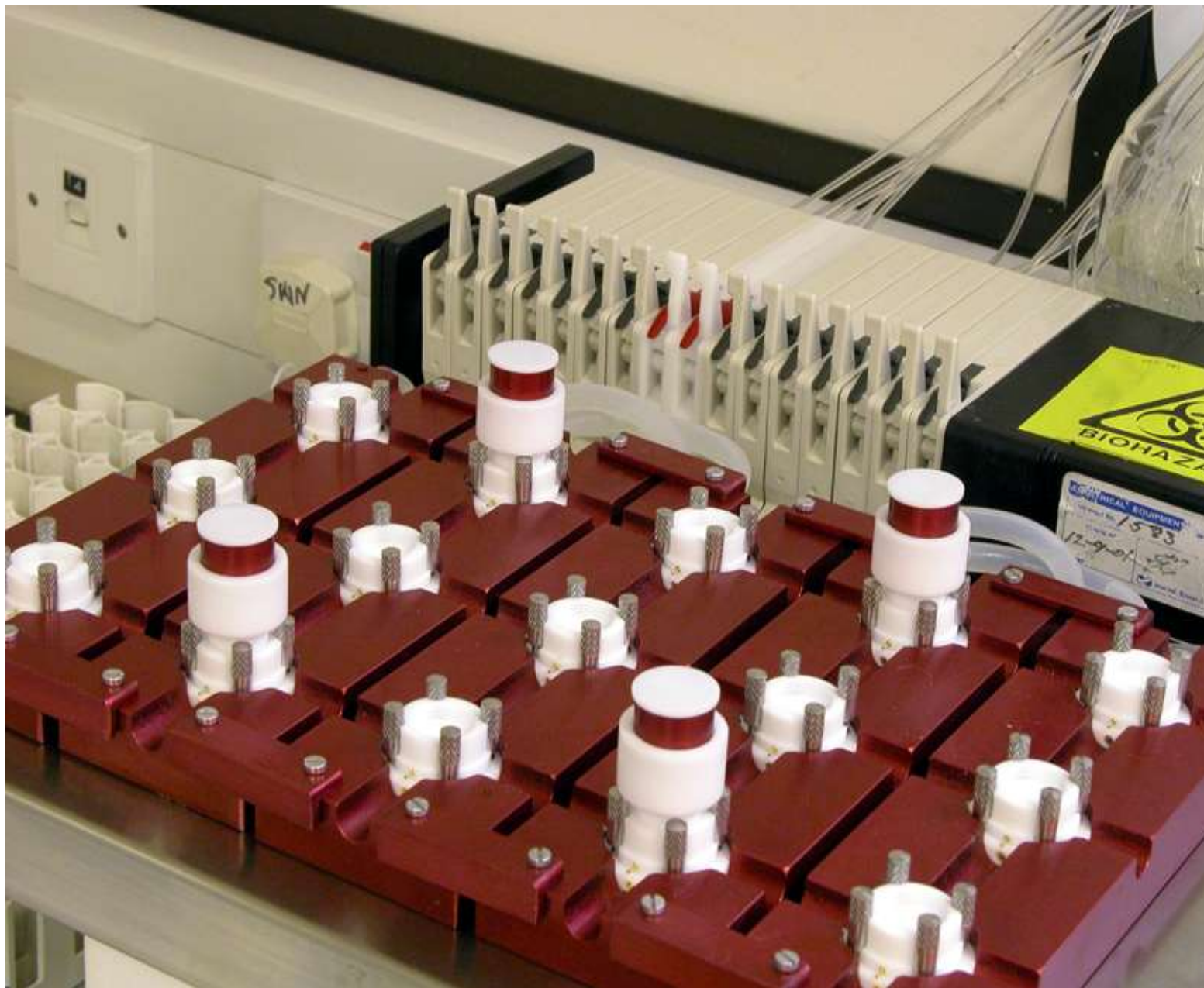
When Can Damage Occur to Skin?

- Patient lifestyle *i.e.* before even going to surgery
- Patient may have skin disease e.g. psoriasis
- At and during surgery
- During transport to the laboratory

- During processing at the laboratory
- During storage
- During the live phase







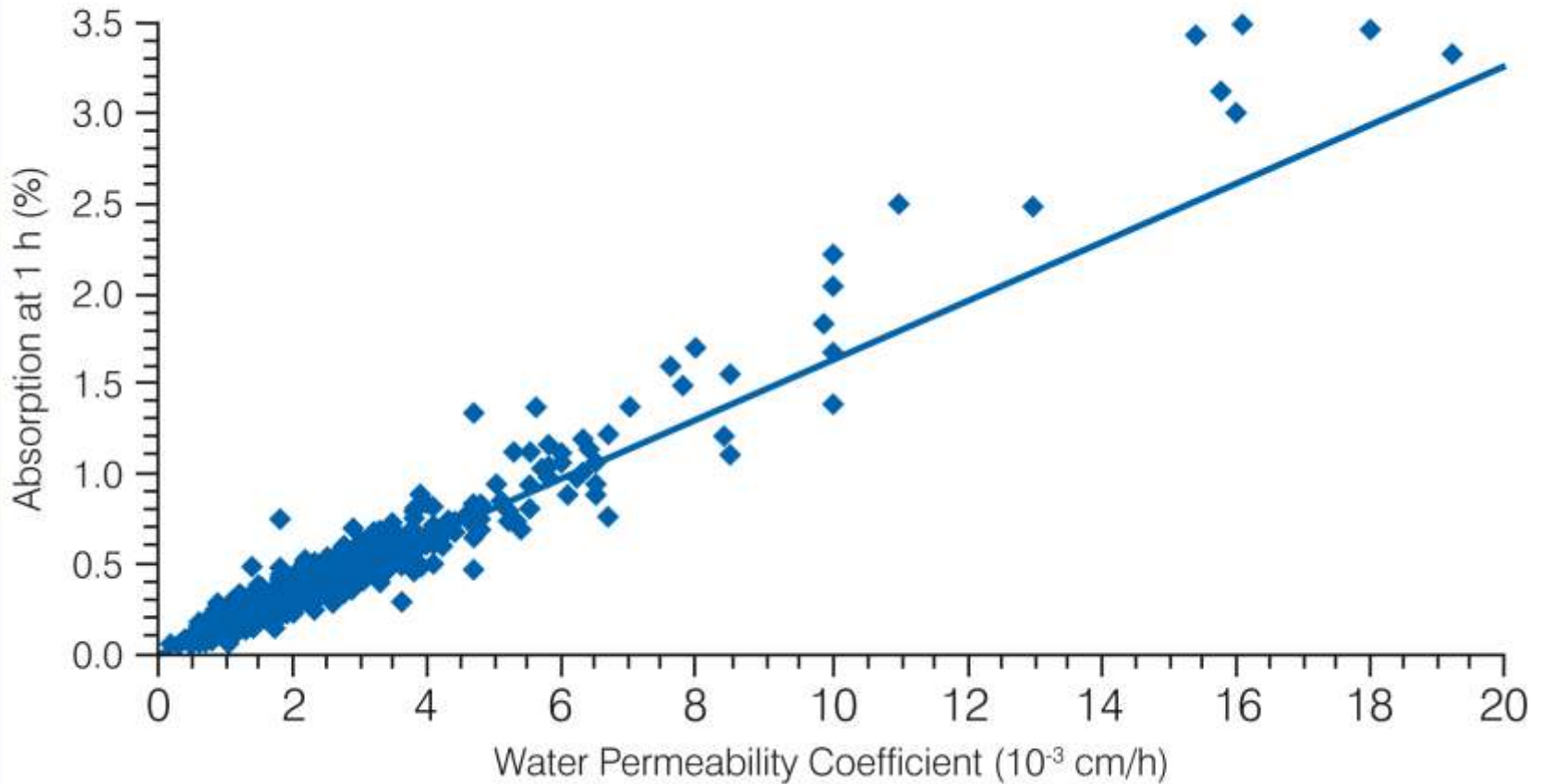
Tritiated Water Barrier Integrity Test

- Place dermatomed skin into cell (0.64 cm²)
- Collect predose receptor fluid
- Apply [³H]-Water (ca 400 μL/cm²)
- Collect mock doses
- Collect receptor fluid at 1 h and 2 h
- Analyse mock doses and receptor fluid by LSC
- Calculate tritiated water k_p or % of applied dose
- Accept/ reject samples

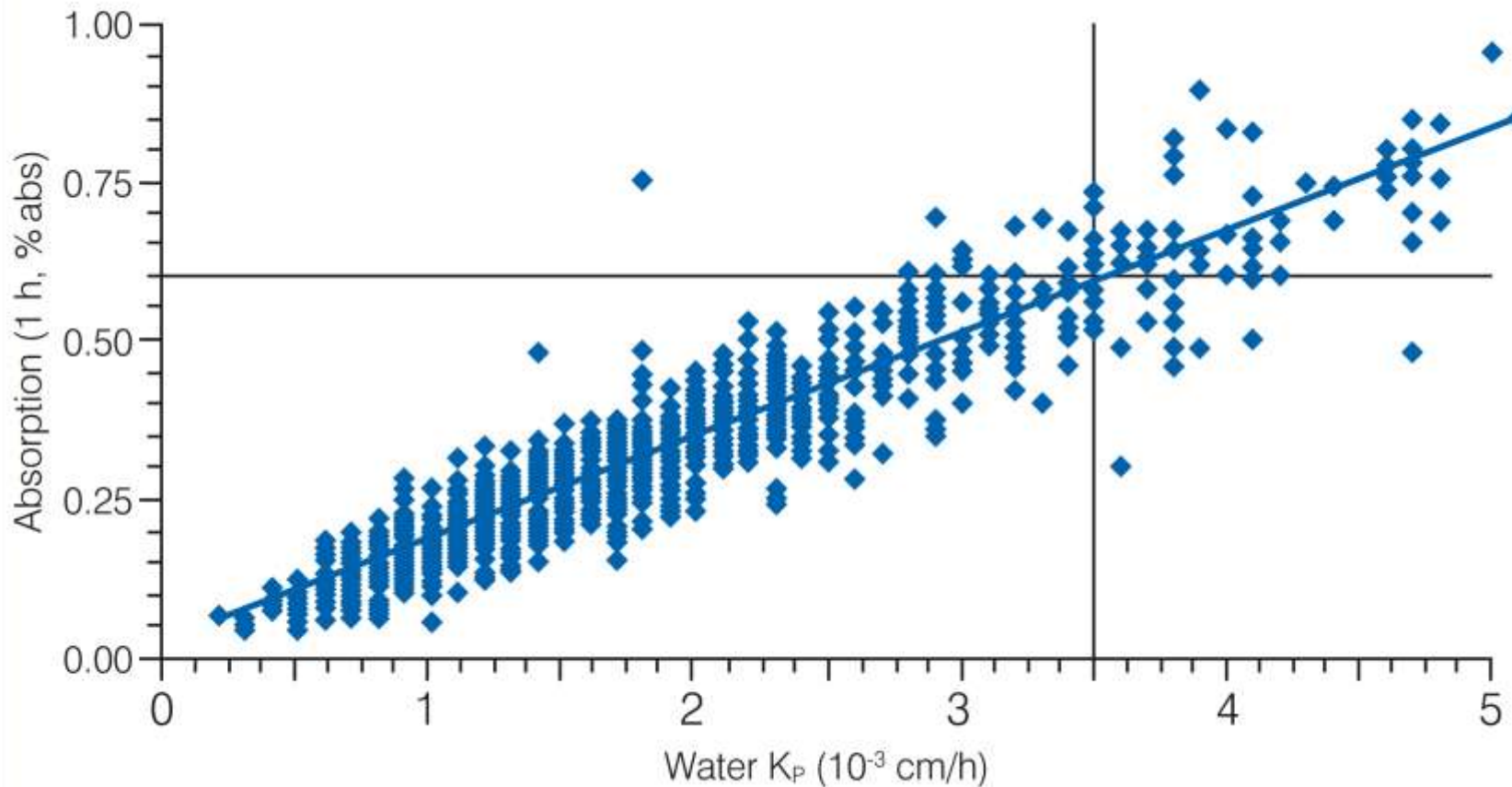
Retrospective Analysis of Samples

- 1082 k_p samples from 25 regulatory toxicology tests
- Human dermatomed skin
- 96 different donors
 - 34 abdomen
 - 60 breast
 - 2 upper arm
- Aged 19 to 87 years
- 2 to 47 replicates from each donor (subject to size of sample supplied)

Results – All 1082 Samples



Results – Water $k_p < 5 \times 10^{-3}$ cm/h



Results – Summary of Acceptability of Test

	Number of Samples	Percentage (%)
False Positives	14	1.29
False Negatives	14	1.29
Both Rejected	93	8.60
Both Accepted	961	88.82
Total	1082	100

Conclusion

- A rejection criterion of 0.6% of the applied dose of tritiated water in 1 h is equivalent to a tritiated water k_p of 3.5×10^{-3} cm/h
- This value has been accepted for use in the regulatory skin penetration studies performed in this laboratory

Acknowledgements

- **Jill Runciman**
- Frank Toner
- Leanne Page
- Craig Blackstock