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Current Status Of A Novel Genotoxicity Assay Using A 3-D Human Skin Model, EpiDerm™

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Background

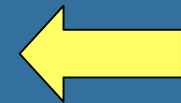
- The 7th Amendment to the Cosmetics Directive prohibits safety testing in animals - *Starting in 2009, in vivo genotoxicity tests for cosmetic ingredients will not be allowed.*
- The REACH program will be conducted using a base of non-animal test methods – genotoxicity is needed.
- Although numerous *in vitro* genotoxicity tests exist, *in vivo* tests are still commonly used.
- None of the existing genotoxicity tests are based on human tissue.



Where Does the Reconstructed Skin Micronucleus (RSMN) Assay Fit?

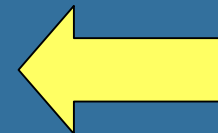
Currently, genotox testing for cosmetics often proceeds:

- Stage 1 – Characterization by existing knowledge, e.g if no dermal absorption, no genotox required
- Stage 2 – Basic in vitro assays
 - Bacterial mutation – Ames
 - Mammalian mutation – Mouse Lymphoma
 - Chromosomal damage – uNucleus or CA
 - Photogenotox, if warranted
- Stage 3 (if any in Stage 2 are positive)
 - In vivo uNuc (bone marrow)
 - In vivo UDS (liver)
 - Point of contact assay (skin generally)



RSMN

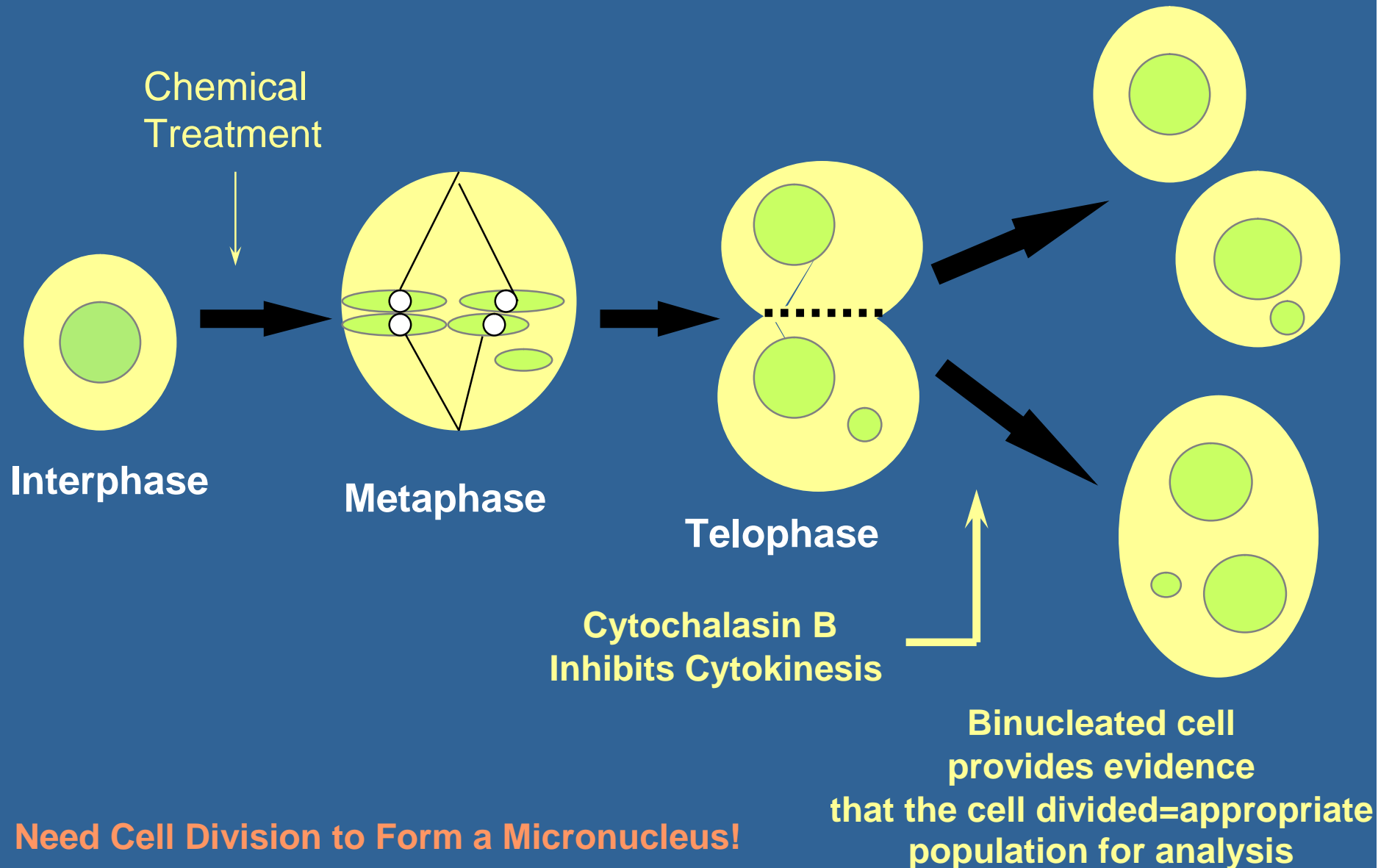
(The most optimistic outcome)



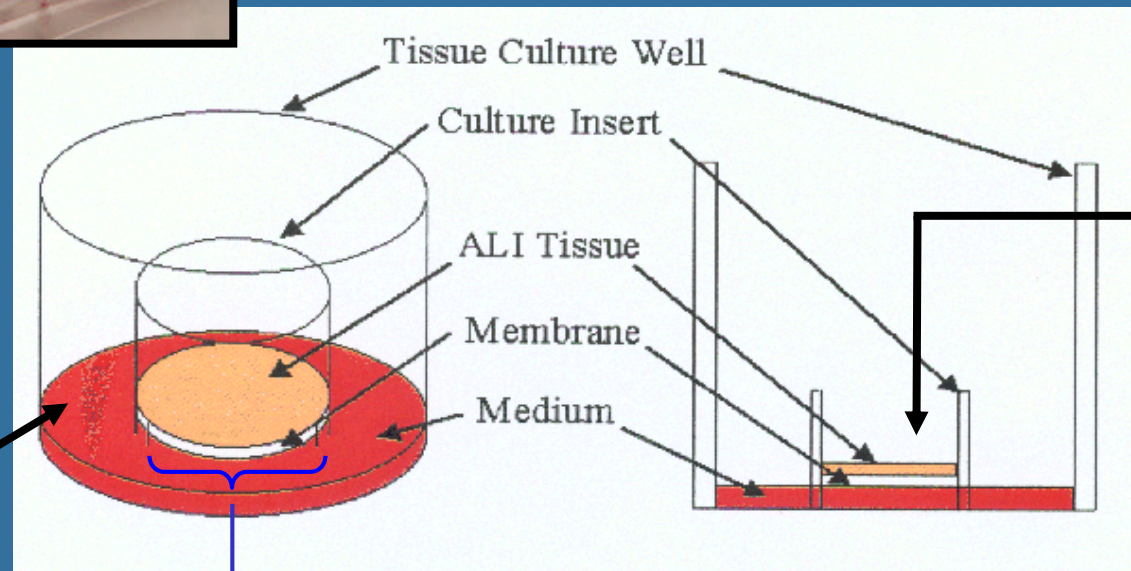
RSMN



In Vitro Micronucleus Assay



EpiDerm Cell Cultures



Exposure to test chemical in media

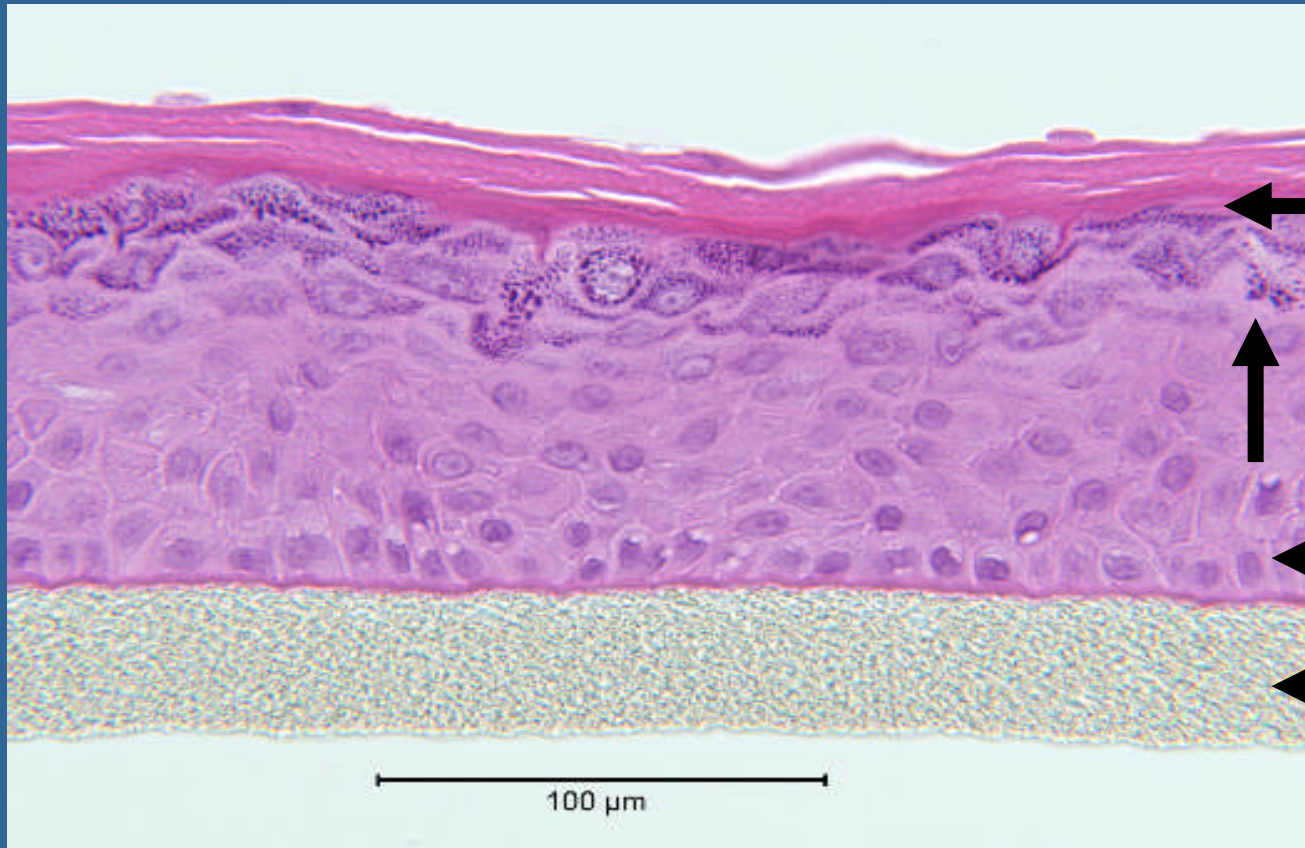
Area = 0.64 cm^2

Exposure topically



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Histology



Stratum corneum;
keratinized dead cells

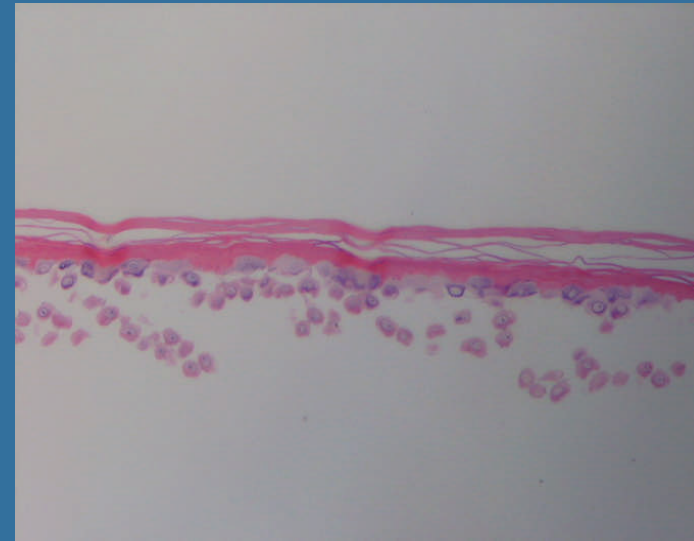
As keratinocytes divide,
they move up and
differentiate

Basal keratinocytes
are dividing cells

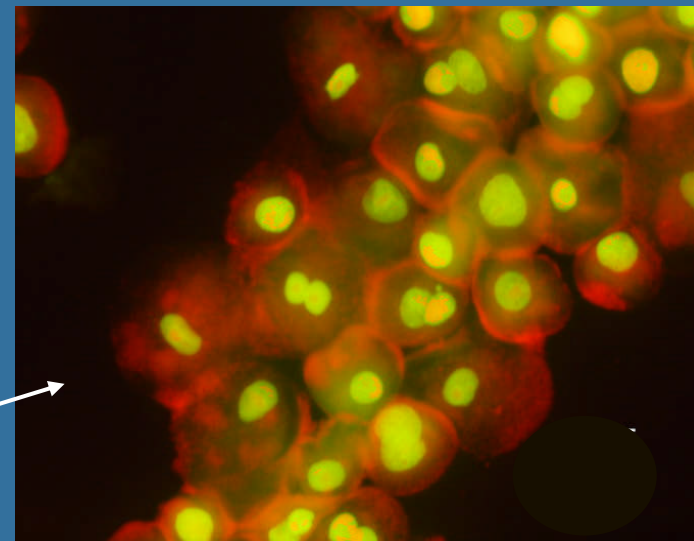
EpiDerm cell
culture insert

Results of Cell Disruption

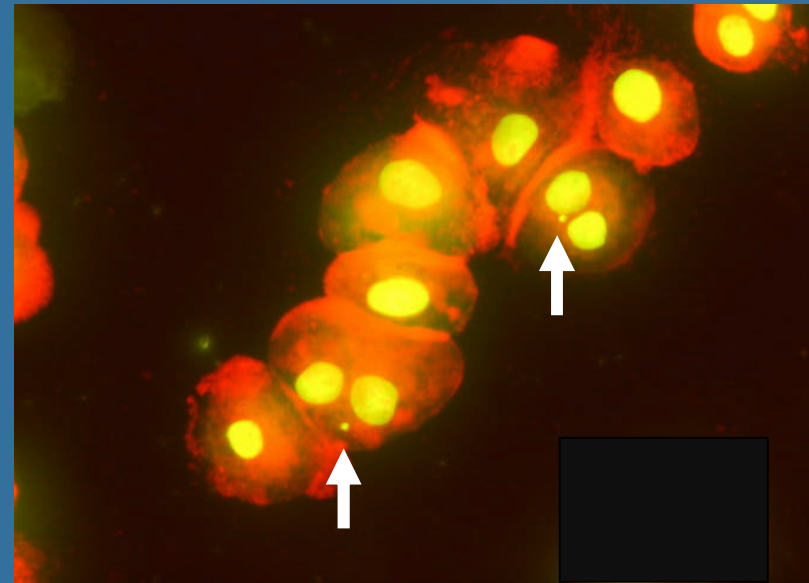
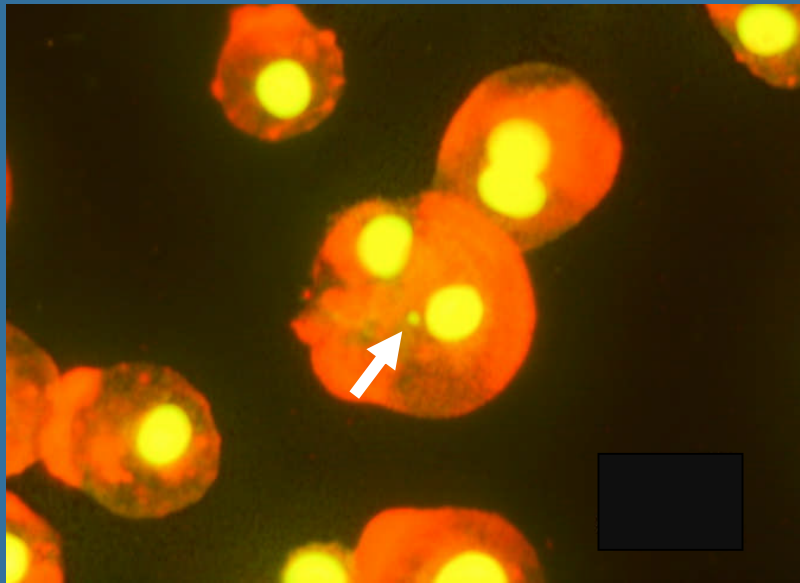
A “pad” of stratum corneum and stratum granulosum remains after trypsinization.



Isolated cell suspension – mostly basal and suprabasal cells – is stained with AO.

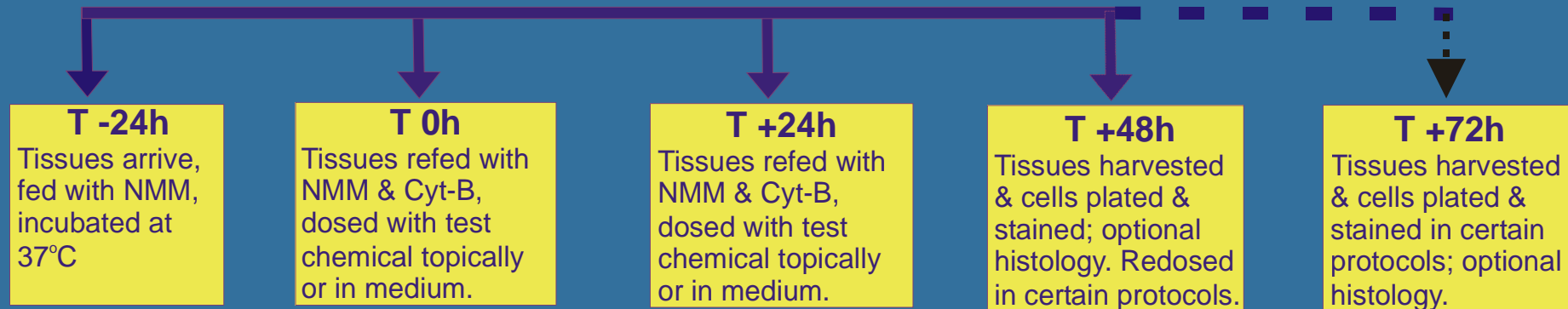


Examples of Micronuclei



Experimental Design

Standard Treatment Protocol



Measurements

Toxicity: % binucleated cells

Genotoxicity: % binucleated cells with micronuclei

3 tissues/treatment, 1000 cells/tissue

Over 75 studies conducted to date

Development of a Method for Assessing Micronuclei Induction in a 3-D Human Skin Model EpiDerm™. Rodger D. Curren, Greg C. Mun, David P. Gibson, and Marilyn J. Aardema, Mut. Res, 607,2006



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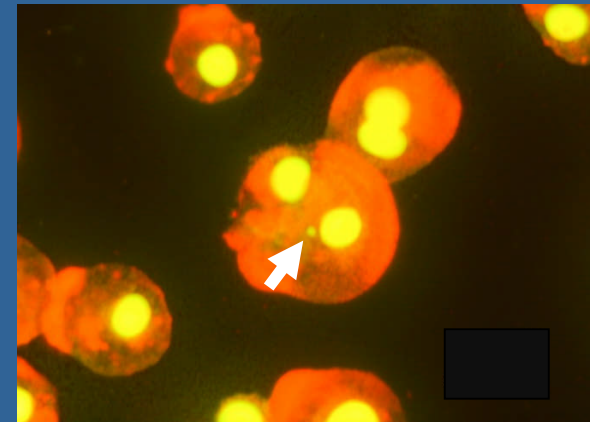
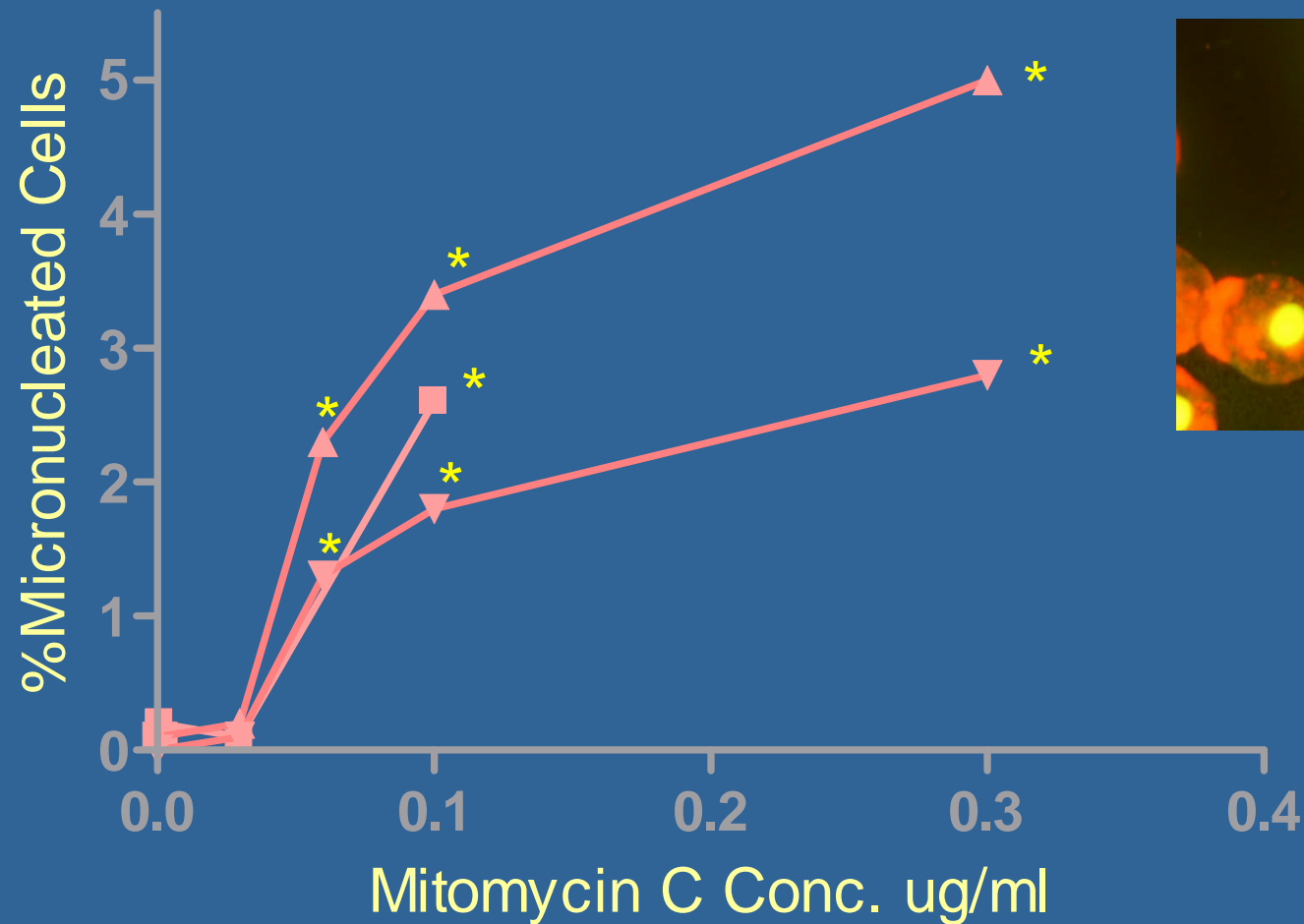
Historical Negative Control Response

EpiDerm cultures treated with
3 ug/ml Cytochalasin B for 24 h,
data through 11/06

- Average % Binucleation = 37.3%; Range 13.8 – 59.4%; 25 Exps.; 64 tissues
- Average MN frequency = 0.1%; Range 0.0 – 0.5%; 25 Exps.; 55 tissues

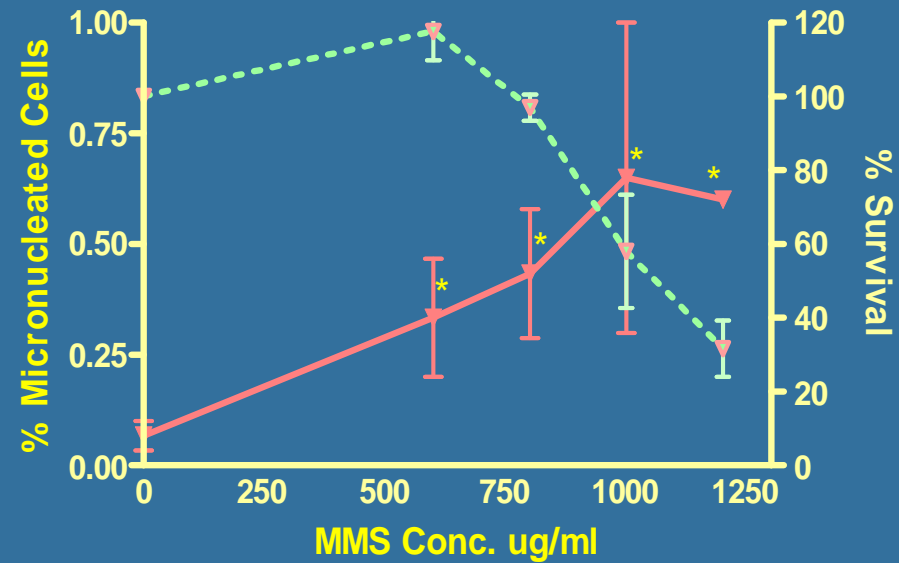
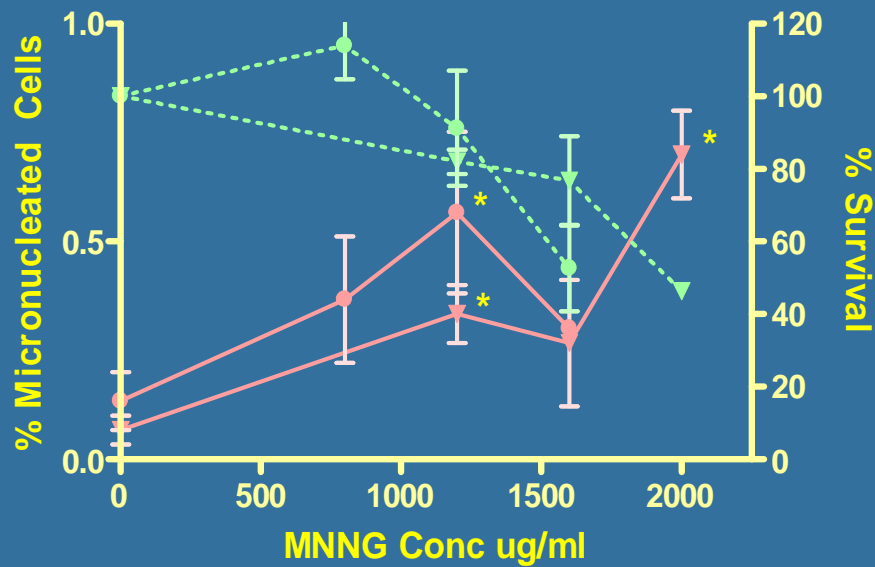


Induction of Micronuclei in EpiDerm, MMC in Media, 48 h harvest

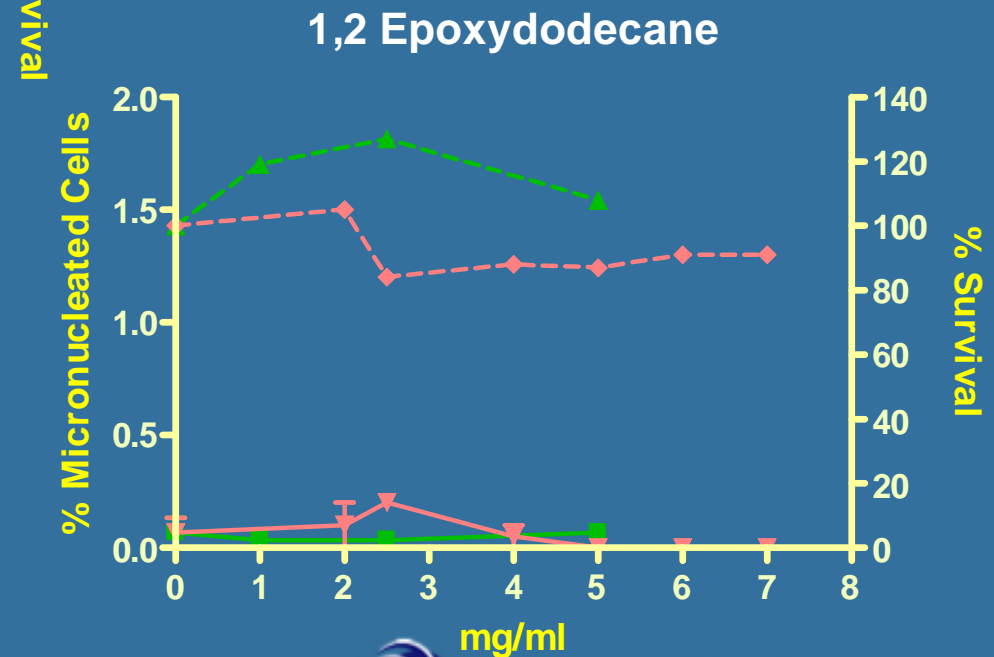
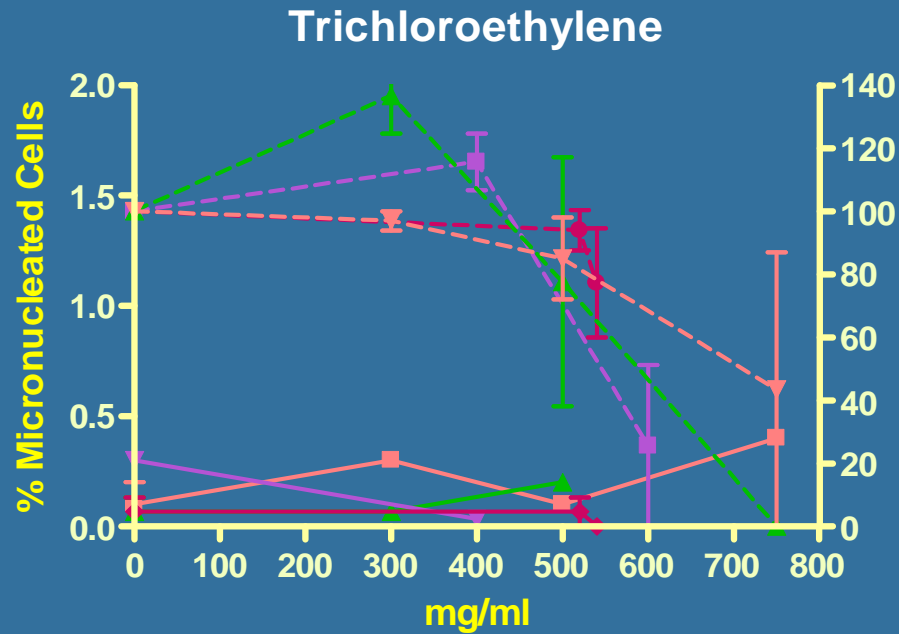


Chemically-Induced Micronuclei Topical Exposure

Direct-acting compounds, positive in in vivo bone marrow
and skin MN



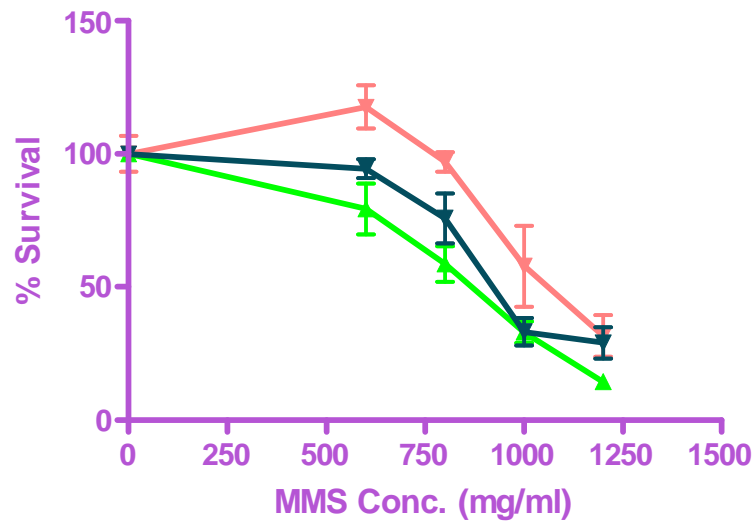
Rodent skin non-carcinogens, negative in in vivo skin MN are negative in EpiDerm™ MN



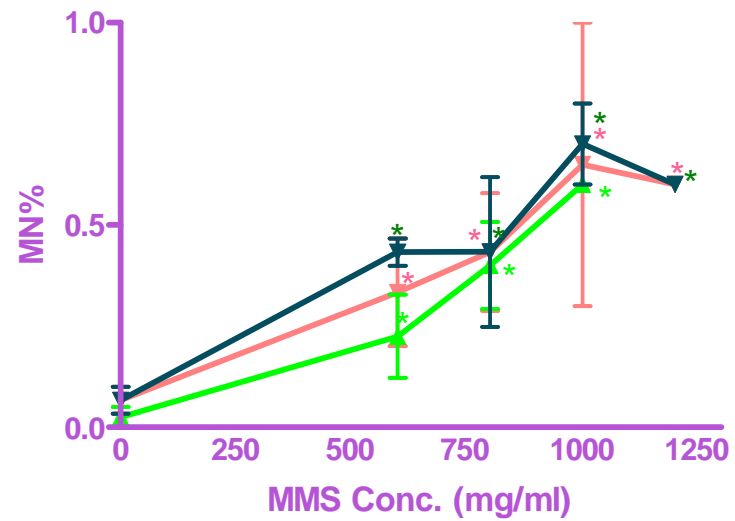
mg/ml

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Between Laboratory Reproducibility Positive Genotoxin (MMS)



—▼ IIVS Donor 254
—▼ PG Donor 254
—▲ PG Donor 926



—▼ IIVS Donor 254
—▼ PG Donor 254
—▲ PG Donor 926



Between Laboratory Reproducibility

Negative Control (acetone) Data

	<u>MatTek</u>	<u>IIVS</u>	<u>P&G</u>
<u>Binucleated Cells:</u>	43.9%	37.3%	40.2%
S.D.	8.4	7.4	10.1
Range	26 - 56%	14 - 59%	22 - 56%
n (experiments)	15	25	7
<u>Micronucleated Cells:</u>	0.07%	0.10%	0.12%
S.D.	0.08	0.1	0.07
MN/1000 BNC	0 - 2	0 - 5	0 - 4
n (experiments)	15	25	7



Metabolic Competence Studies

17 /17 CYP Genes Agreed between Normal Human Skin and EpiDerm™

Gene Symbol	Normal Human Skin	Wellcome EpiDerm
CYP1A2	P	P
CYP2A6	P	P
CYP2B6	A*	A*
CYP2C8	A*	A*
CYP2C9	P	P
CYP2C18	P	P
CYP2C19	P	P*
CYP2F1	A	A
CYP2J2	P	P
CYP3A5	P*	P*
CYP3A7	A*	A*
CYP4B1	P	P
CYP4F3	P	P
CYP11B1	P	P
CYP17A1	A	A
CYP24A1	A	A
CYP51A1	P	P

Collaboration
between P&G,
MatTek &
Oxford Univ.

*: Genes confirmed by RT-PCR.

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Five CYP Genes with Different Calls

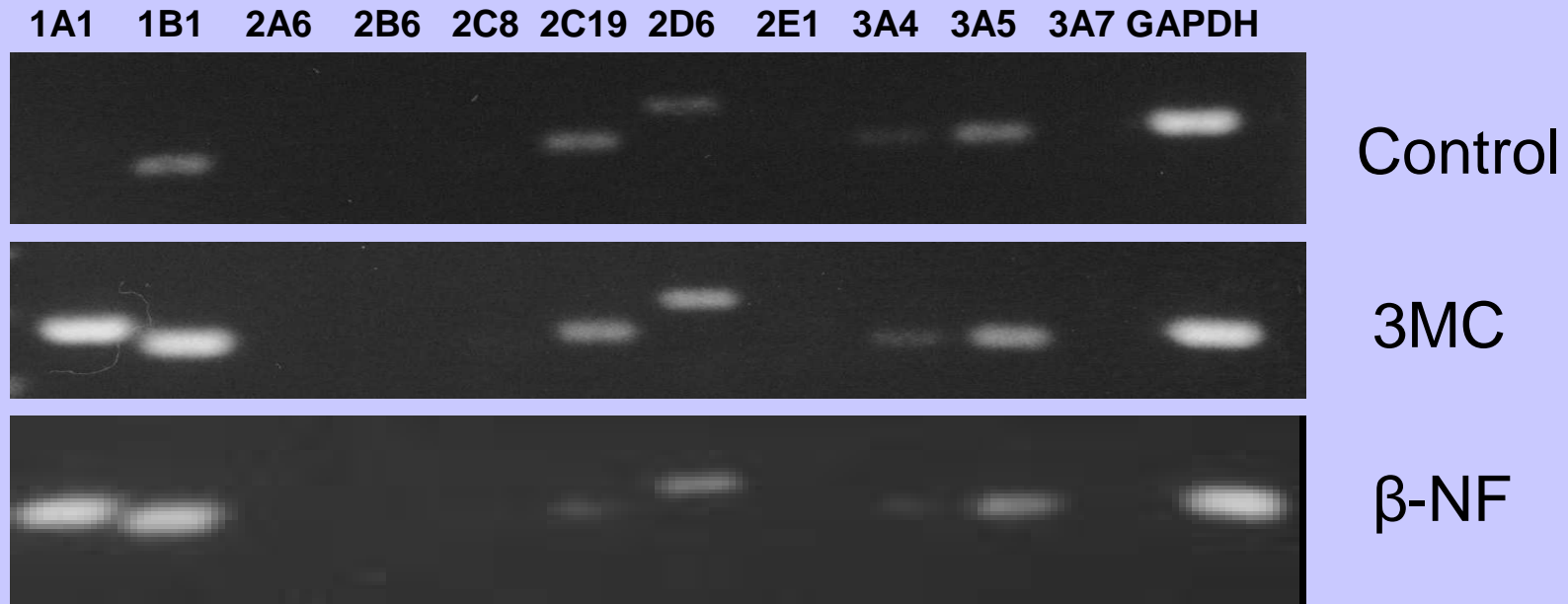
Gene Symbol	Normal Human Skin	Wellcome EpiDerm
CYP1A1	P	A*
CYP1B1	P*	A (P*)
CYP2D6	P*	A (P*)
CYP2E1	P	A
CYP3A4	P*	A (P*)

*: Genes confirmed by RT-PCR

Overall: 20/22 CYP genes agreed between normal human skin and EpiDerm™ Cultures.



RT-PCR Results for CYP Inducibility in EpiDerm™



Inducible with 3MC: 1A1, 1B1, 2C19, 2D6, 3A4, 3A5

Inducible with β -NF: 1A1, 1B1, 2D6, 3A5

EpiDerm™ model appears to be metabolically competent

Chemical results confirmed in at least two laboratories

Positive Genotoxins

- MMC
- VB
- Cyclophosphamide*

* = Requires metabolic activation; 72 h exposure protocol required

Positive Dermal Carcinogens

- MNNG
- MMS
- ENNG
- BBL
- DCC
- MNU
- ENU

Negative Dermal Non-Carcinogens

- 4-nitrophenol
- 1,2 epoxydodecane
- trichloroethylene
- 2-ethyl-1,3-hexanediol
- 2-PP



International Efforts For RSMN Investigations

- Special workshop on Genetox in reconstructed skin models convened by ZEBET
 - Invited representatives from skin model manufacturers, SCCP, Federal Drug Agency and academics
- Internal funding from P&G and IIVS for preliminary studies
- Colipa - funded program (5 laboratories) for both MN and COMET assay in several 3D skin models. ECVAM additional funding and involvement.
- Small Business Innovative Research (SBIR) Phase I contract from US Government to MatTek Corporation.



Conclusions

- EpiDerm™ 3-D reconstructed human skin has essential properties to allow its use in a MN assay.
- Initial studies show that 2 known direct-acting genotoxins and 7 rodent dermal carcinogens are positive in the RSMN, and 5 known rodent skin non-carcinogens are negative.
- Results with cyclophosphamide (3 exposures, 72 h protocol) which requires metabolic activation are positive. First human model to show positive genotoxicity results.
- Because of its potential value to address 7th amendment testing bans (and its potential to provide labeling information for REACH), continued development should be encouraged



Acknowledgements

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