Safety assessment of topically administered pharmaceuticals with special emphasis on the minipig

PRECLINICAL SCIENCE

Dr. Lars Mecklenburg

DVM, PhD, DACVP

Relevance of Dermatotoxicology

PRECLINICAL SCIENCE

- Accidental occupational or environmental exposure
  - Chemicals
  - UV light
- Intentional application to skin
  - Cosmetics
  - Consumer products
  - Drugs

Dermal safety testing

PRECLINICAL SCIENCE

- Skin **absorption**
  - in vitro (OECD 428)
  - In vivo (OECD 427)
- Skin **irritation** / corrosion
  - In vitro (OECD 430, 431, 435)
  - In vivo (OECD 404)
- Skin **sensitization**
  - Maximisation test / Buehler test in guinea pigs (OECD 406)
  - Local lymph node assay in mice (OECD 429)
- Dermal **toxicity, single administration** (OECD 402, 434)
- Dermal **toxicity, repeated administration** (OECD 410, 411)
- **Phototoxicity** / photosensitization
  - Photoirritation (OECD 432)
  - Photocarcinogenicity (enhancement of UV-induced skin carcinogenesis)

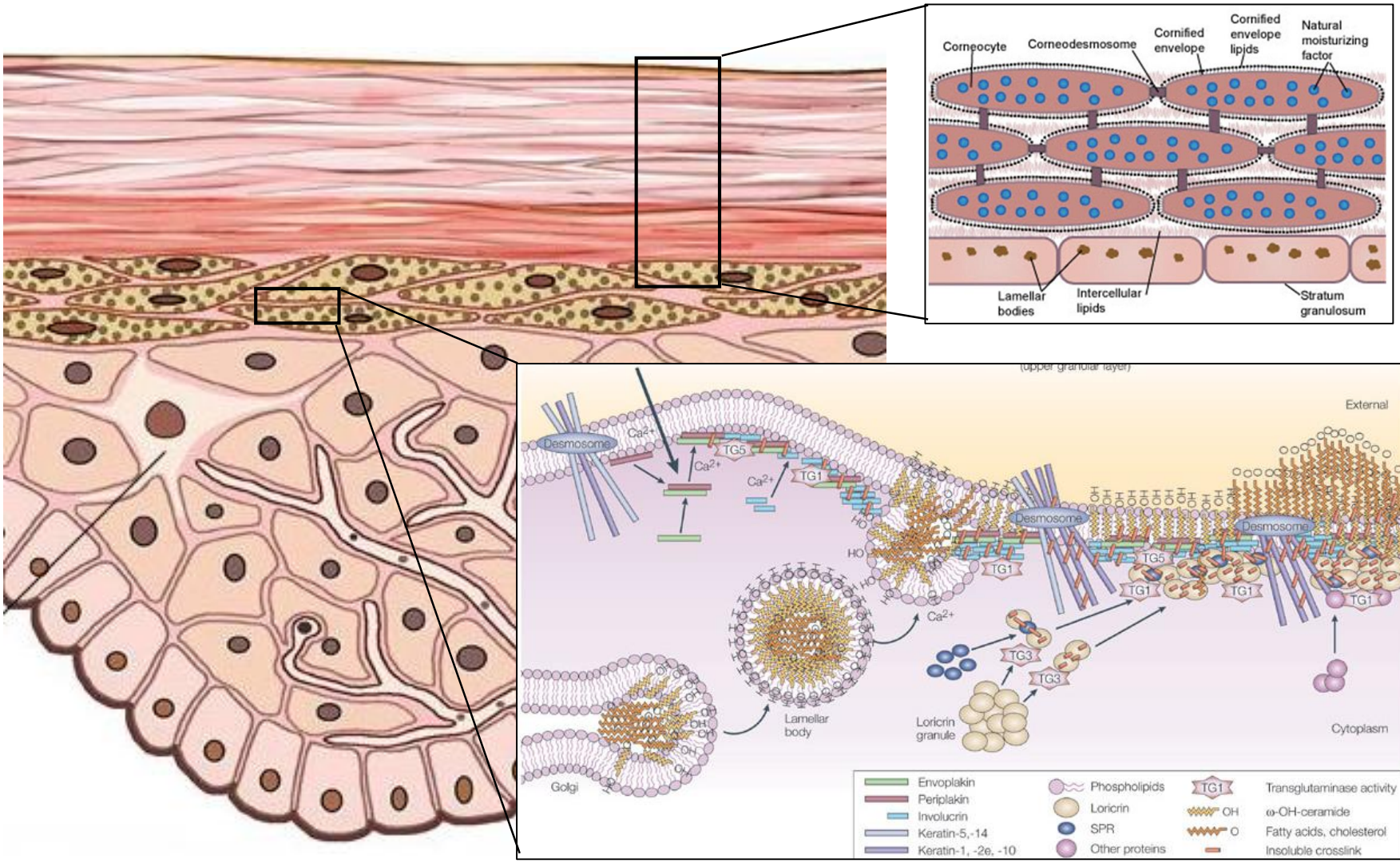
Percutaneous absorption

PRECLINICAL SCIENCE

- Ability of the API to leave its vehicle and penetrate into the epidermis (or beyond)
- Stratum corneum most limiting to absorption
  - One of the most important functions of the epidermis is to limit free passage of fluids and electrolytes from the underlying tissue to the environment
  - Acquired as an evolutional adaptation for survival in non-aquatic conditions
  - Limits entry of harmful chemicals into the body
    - K_{WO} of Stratum corneum = $1.1 * 10^{7}$ cm/sec
    - K_{WO} of Dermis = $1.0 * 10^{4}$ cm/sec
- Water content depends on air humidity; Stratum corneum can absorb up to 6 times its own weight in water and will increase its thickness 3 times
- Diffusion through the Stratum corneum is purely passive

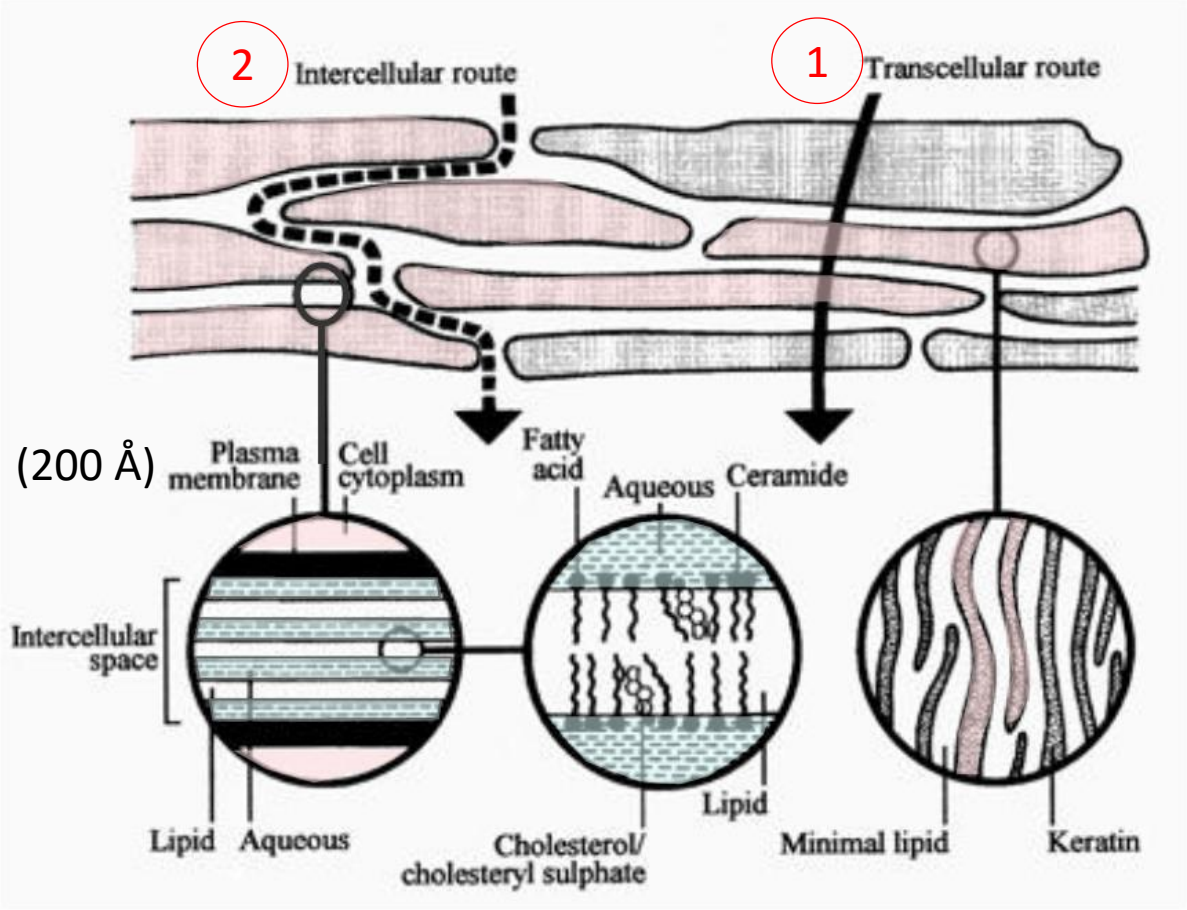
Stratum corneum

PRECLINICAL SCIENCE



Percutaneous absorption

PRECLINICAL SCIENCE



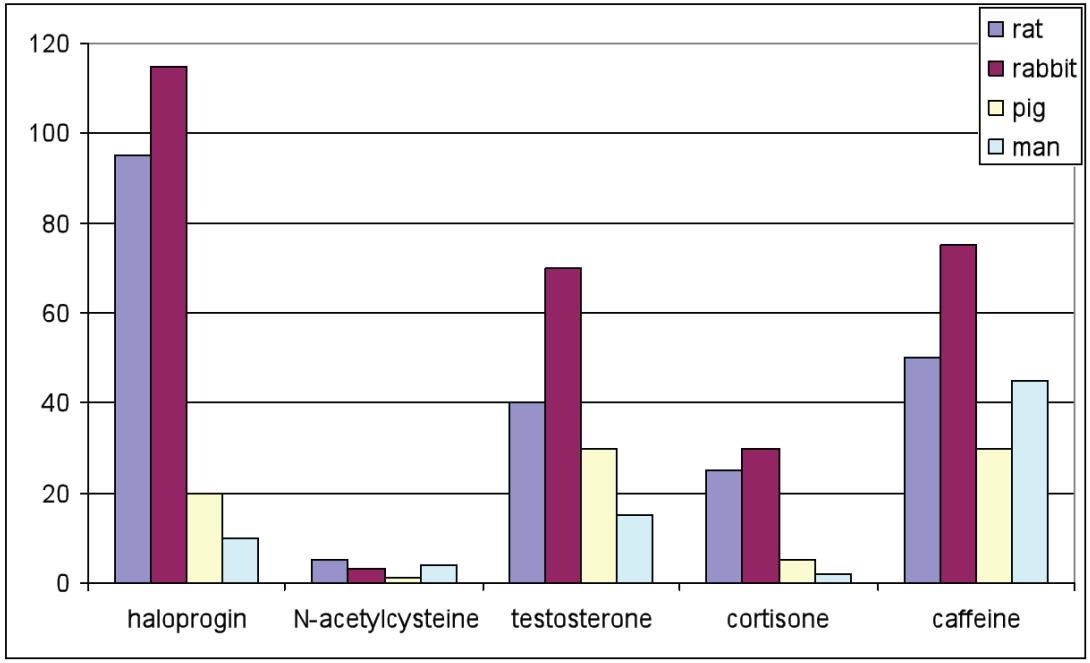
40% water
40% protein
20% lipid

3 Diffusion through pilosebaceous units and sweat glands

Percutaneous absorption

PRECLINICAL SCIENCE

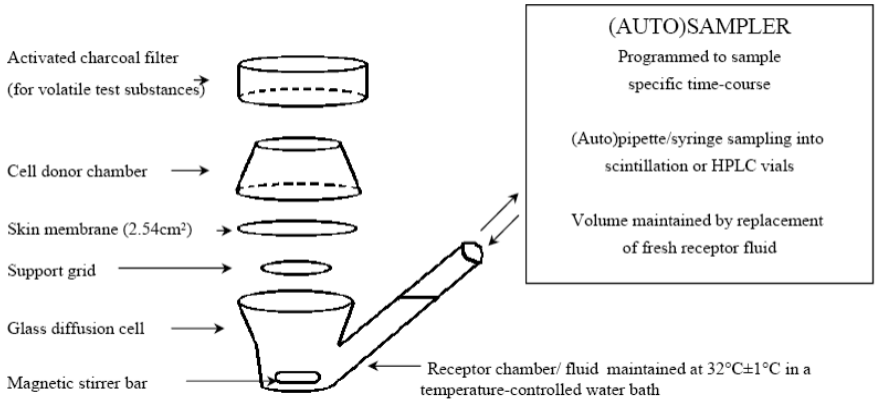
- Air humidity
- Mass
- Concentration
- Time of exposure
- Exposed surface area
- Vehicle
- Skin condition
- Anatomy (species)



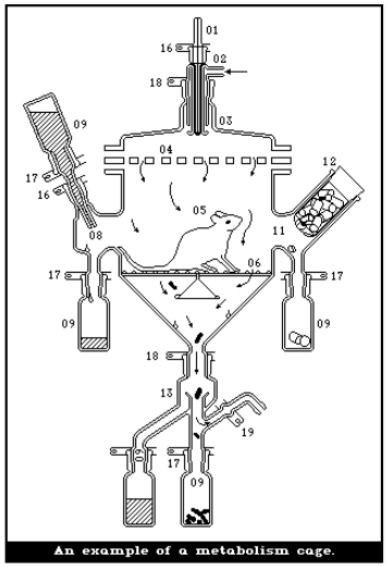
Percutaneous absorption

PRECLINICAL SCIENCE

- in vitro (OECD 428)
  - Diffusion of test article into and across human skin



- in vivo (OECD 427)
  - Rat
  - Individually housed in metabolism cage
  - Hair clipping on the dorsum
  - Application of the test article ($1 – 5$ mg/cm^2)
  - Occlusion
  - $6 – 24$ hrs exposure
  - Necropsy and analysis of organs


An example of a metabolism cage.

Metabolic competence

PRECLINICAL SCIENCE

- Most drug metabolizing enzymes also occur in the mammalian skin albeit at relatively low specific activities *(Oesch et al. 2007; Oesch et al. 2014)*
  - 10 – 20% of the metabolic competence of the liver
  - Mostly localized in suprabasal epidermal layers
- In skin, cytochrome P450 enzymes (CYP) protein levels are 300-fold lower compared to liver *(van Eijl et al. 2012)*
  - Largest group of xenobiotic metabolizing enzymes that catalyze mono-oxygenation by transferring one oxygen atom onto the substrate
  - Expression varies across species, age, gender, and organ
  - Transcripts of CYP1A1/2/6, CYP 1B1, CYP2C9, CYP2D6, CYP2E1, CYP3A4/5 shown in native human skin *(Hu et al. 2010; Hayden et al. 2006)*
- Activities of non-CYP oxidoreductases are relatively more relevant for xenobiotic metabolism in skin (4-10 fold lower protein expression compared to liver)
  - Flavin-dependent monooxygenases (FMO)
  - Cyclooxygenases (COX)
  - Alcohol dehydrogenase (ADH)
  - Hydrolases and phase II enzymes are well presented in skin

Archives of Toxicology (2018) 92:2411–2456
https://doi.org/10.1007/s00204-018-2232-x

REVIEW ARTICLE

PRECLINICAL SCIENCE

Xenobiotica-metabolizing enzymes in the skin of rat, mouse, pig, guinea pig, man, and in human skin models

F. Oesch[1] · E. Fabian[2] · Robert Landsiedel[2]

Table 1 Representative Cytochrome P450 (CYP) basal activities in skin microsomes of various mammalian species

Activity (preferential for)	Human	Rat	Mouse	Guinea pig	Pig
AHH (CYP1 family)	0.24 to 1.35[a]	1.25 ± 0.11[a]	m: 3.3–46[a]; f: 17–21[a]	2.51 ± 0.35[a]	
EROD (CYP1 family)	bd to 35[a]	m: 3.6 ± 0.3; f: 1.5 ± 0.2[a]	m: bd; f: 3–19[a]		4.62 ± 0.54[b]
ECOD (CYP1A,1B,2B,2D6,3A4)	bd to 12[a]	0.36–2.15[a]	10.4–80[a]	3.8 ± 2.7[a]	(13.2 ± 2.5[b])
MROD (CYP1A2)	bd to +				
PROD (CYP2B)	bd to bq	m: 3.7 ± 1.3; f: 1.8 ± 0.1[a]	m: bq; f: 0.1–1.7[a]	bq	bd
BROD (CYP3A, 2B)		m: 4.4 ± 0.9; f: 2.1 ± 0.2			
Aminopyrine *N*-demethylase (CYP2B, 3A)		1000–4200[a]	+		
Tolbutamide 4-hydroxylation (CYP2C9)	0.46 ± 0.05[b]	0.47 ± 0.04[b]	bd		1.66 ± 0.49[b]
Bufuralol 1-hydroxylation (CYP2D6)	bd	1.33 ± 0.17[b]	9.23 ± 0.67[b]		0.26 ± 0.03[b]
Chlorzoxazone 6-hydroxylation (CYP2E1)	2.83 ± 0.34[b]	bd	20.8 ± 0.5[b]		bd
Para-nitrophenol hydroxylation (CYP2E1)	bd/+	bd[c]	f: 40 ± 10[a]		
Midazolam 1-hydroxylation (CYP3A)	2.35 ± 0.23[b]	0.58 ± 0.09[b]	8.70 ± 0.28[b]		2.32 ± 0.21[b]
Benzoquinoline O-dealkylation (CYP3A)	bd to 76 ± 41[a]				
Erythromycin N-demethylation (CYP3A)	+	bd–270[a]	f: 540–1100[a]		
Testosterone (CYP2A1, 3A4, 19A1, 2C11, 2C19)[d]					+
BP 7,8-dihydrodiol (CYP1A/1B, 2S1)	+	+	+		

More examples and references in the text

AHH aryl hydrocarbon hydroxylase, phenolic benzo[*a*]pyrene metabolites determined with 3-hydroxybenzo[*a*]pyrene as standard, *bd* below detection, *BP* benz[*a*]pyrene, *BROD* 7-benzyloxyresofufin *O*-debenzylase, *bq* below quantification, *ECOD* 7-ethoxycoumarin-*O*-deethylase, *EROD* 7-ethoxyresorufin-*O*-deethylase, *f* female, *m* male, *MROD* 7-methoxyresorufin-*O*-demethylase, *PROD* pentoxyresorufin *O*-dependentylase

[a]pmol/mg protein/min

[b]pmol/mg protein/h, numbers in brackets: in medium of short-term culture

[c]In epidermal microsomes

[d]Indication of the authors (Jacques et al. 2014) that all these CYPs are present in their pig ear skin model

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REVIEW ARTICLE

PRECLINICAL SCIENCE

Xenobiotica-metabolizing enzymes in the skin of rat, mouse, pig, guinea pig, man, and in human skin models

F. Oesch[1] · E. Fabian[2] · Robert Landsiedel[2]

Table 2 Representative non-CYP-mediated oxidoreductase activities in skin of various mammalian species

Model substrate (for)	Human	Rat	Mouse	Guinea pig
Benzydamine (FMO)	+			
Methimazole (FMO)			0.35[a]	
Thiobezamide (FMO)			0.32[a]	
Arachidonic acid (COX)	23.5 ± 8.7[b]			
Ethanol[c] (ADH)	0.3–0.4[d]	2.06[d]	1.1–1.2[d]	0.6[d]
2,6-Dichlorophenolindophenol (NQR)	~ 375[d]		23.4 ± 0.8–159 ± 20[d]	
Menadione (NQR)	7–10[d]	+		
Carbazeran (AO)	1.30[e]			
Zoniporide (AO)	0.164[e]			

More examples and references in the text; only constitutive activities

ADH alcohol dehydrogenase, *AO* aldehyde oxidase, *COX* cyclooxygenase, *FMO* flavin-dependent monooxygenase, *NQR* NADH/NADPH quinone reductase

[a]nmol product/mg microsomal protein/min

[b]pg PGE2 formed/mg microsomal protein/min

[c]Beside ethanol ADH activity shown in human skin for 2-butoxyethanol > 2-phenoxyethanol > ethylene glycol > 2-ethoxyethanol as substrates

[d]nmol product/mg cytosolic protein/min

[e]pmol/h/mg skin

OPEN ACCESS Freely available online

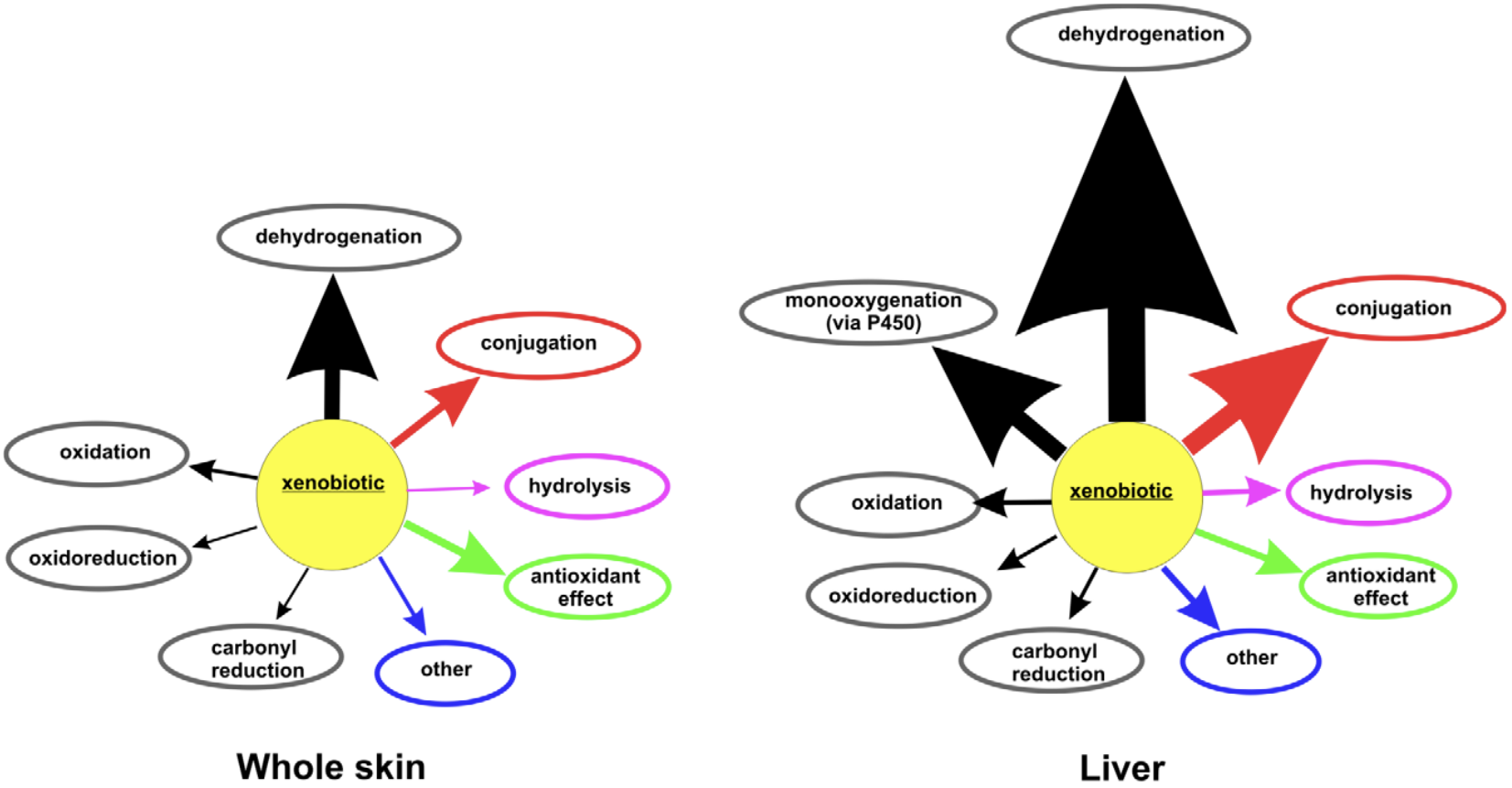
PLoS one

Elucidation of Xenobiotic Metabolism Pathways in Human Skin and Human Skin Models by Proteomic Profiling

PRECLINICAL SCIENCE

Sven van Eijl[1], Zheying Zhu[1], John Cupitt[1], Magdalena Gierula[1], Christine Götz[2], Ellen Fritsche[2,3], Robert J. Edwards[1]*

1 Centre for Pharmacology and Therapeutics, Division of Experimental Medicine, Imperial College London, London, United Kingdom, **2** Leibniz Institut für Umweltmedizinische Forschung, Heinrich-Heine-Universität, Düsseldorf, Germany, **3** Department of Dermatology and Allergology, University Clinic RWTH, Aachen, Germany



Size of each arrow is proportional to the number of enzymes detected

Consequences of skin metabolism

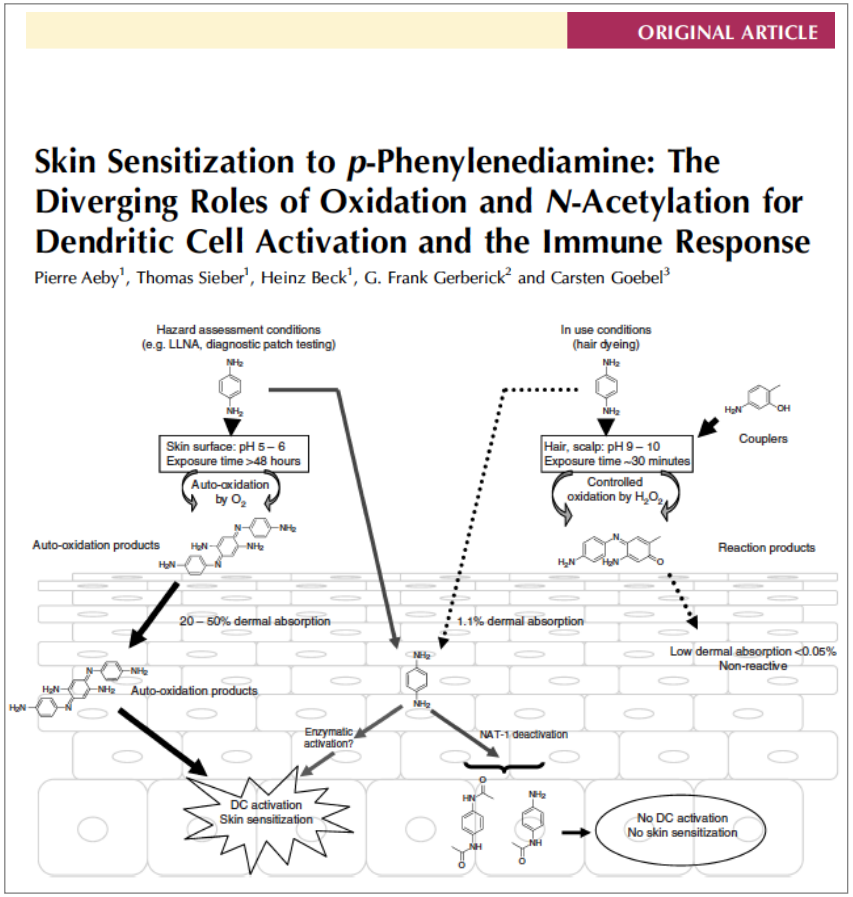
PRECLINICAL SCIENCE

- Hydrolases and phase II enzymes
  - May reduce the size of xenobiotics and convert lipophilic esters into hydrophilic alcohols, thereby **increasing the absorption** of some xenobiotics
- Carboxylesterases
  - Hydrolyze carbon esters by intra-molecular addition of water, **converting non-irritant esters into irritant alcohols**

Pro-sensitization

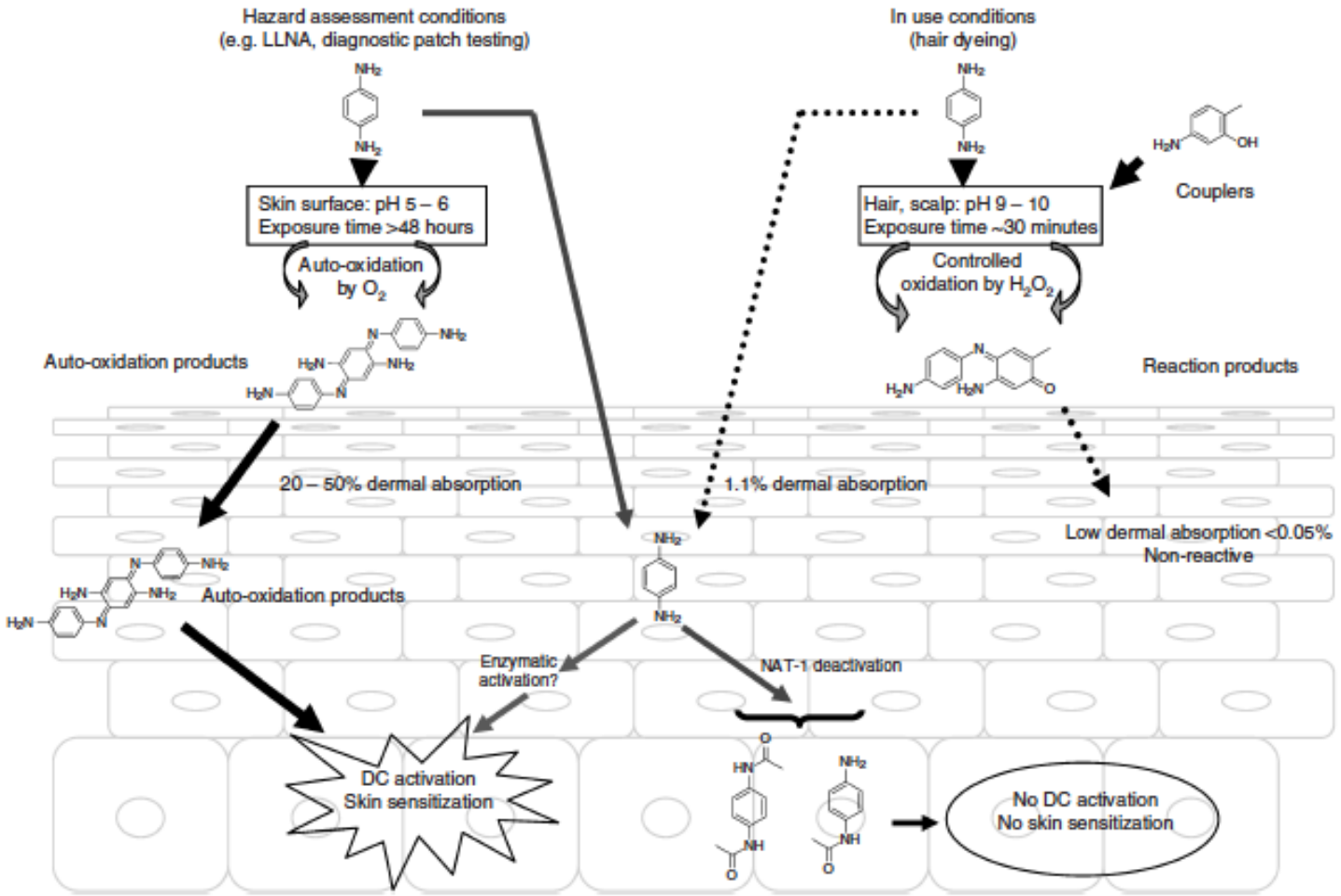
PRECLINICAL SCIENCE

- Some hapten sensitizers are reactive per se, whereas others (pro-sensitizers/ pro-haptens) require activation by auto-oxidation or enzymatic transformation *(Jäckh et al. 2012)*
- Examples
  - Oxidation of cinnamic alcohol into immune-reactive cinnamic aldehyde *(Cheung et al. 2003)*
  - **P-Phenylendiamine:** aromatic amine in hair dyes (activated by auto-oxidation under experimental conditions; detoxification by acetylation in keratinocytes)



Pro-sensitization

PRECLINICAL SCIENCE



Metabolic competence

PRECLINICAL SCIENCE

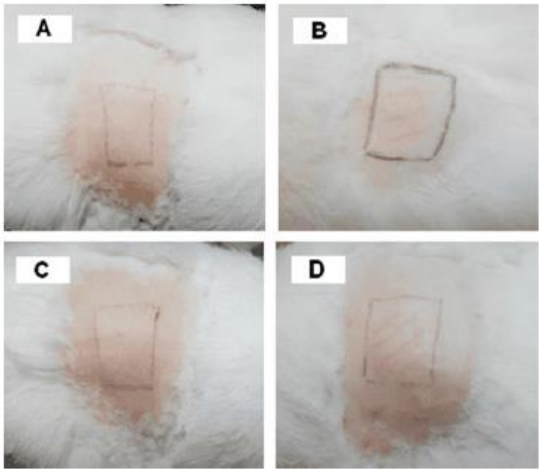
- Consideration of cutaneous metabolism is important for attempting correct predictions of skin irritation in human (particularly relevant for esters)
- The preclinical model should preferably be close to human skin with respect to esterases, alcohol dehydrogenases and aldehyde dehydrogenases
- Esterases
  - pig appears to be a particularly good model
  - rat skin less comparable to humans
  - insufficient information on the skin of mouse and guinea pig
  - all three-dimensional human skin models are good models
- Alcohol dehydrogenase and aldehyde dehydrogenases
  - Rat, mouse and guinea pig skin are reasonably close to human skin

Skin irritation

PRECLINICAL SCIENCE

- In vitro
  - Transcutaneous electrical resistance (OECD430)
  - Human 3D skin model (OECD 431)
  - Membrane barrier test (OECD 435)

- In vivo (OECD 404)
  - Albino Rabbit, individually housed (initial: 1 animal, confirmatory: 2 animals)
  - Clipping hair on the dorsum
  - Test substance applied to ~ 6 cm^2 of dorsal skin
  - 0.5 ml (liquids) or 0.5 g (solids or pastes)
  - Covered with gauze patch
  - 4 hour exposure
  - Observation period: 14 days
  - Macroscopic score at 1, 24, 48, 72 h
  - Histopathology, if neccessary



Irritant vs. Allergic contact dermatitis

PRECLINICAL SCIENCE

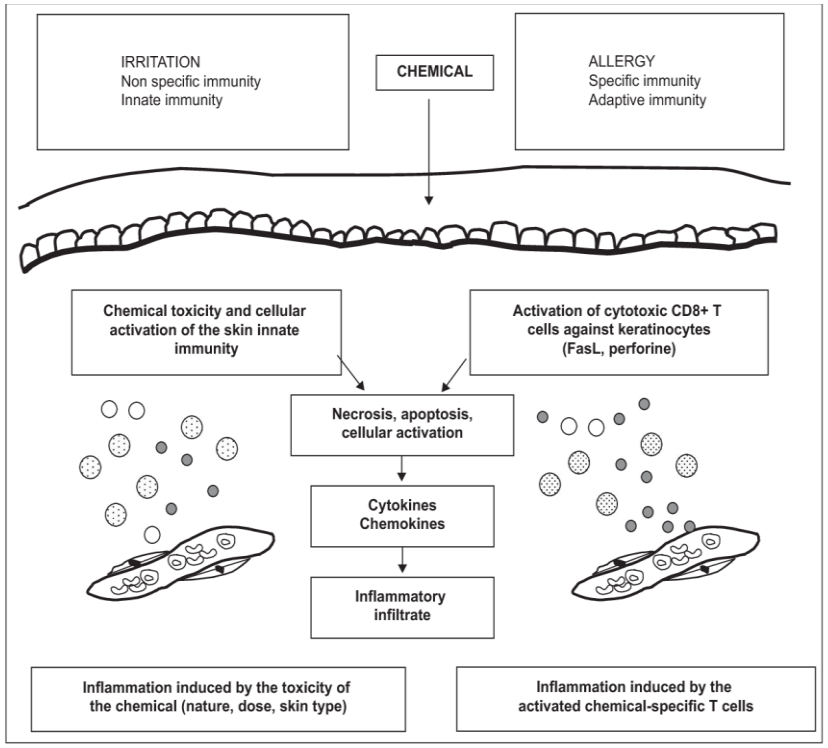
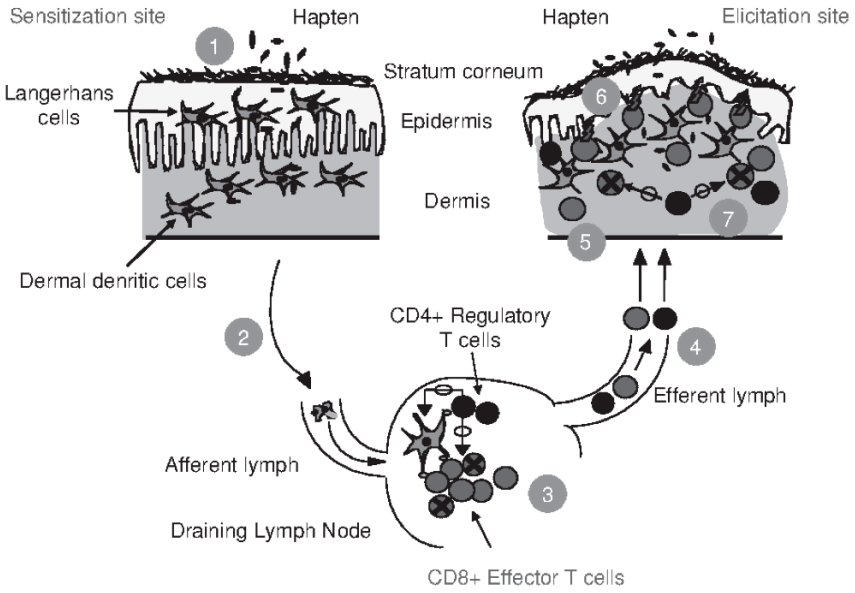


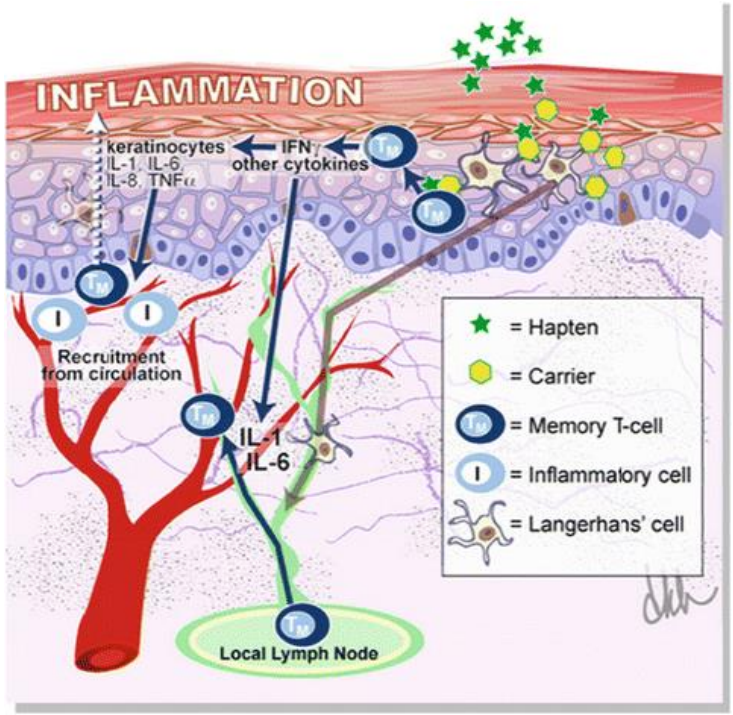
Figure 1. Immune mechanisms in ICD and ACD. ICD and ACD are induced by skin contact with chemicals. The early stage



Skin sensitization

PRECLINICAL SCIENCE

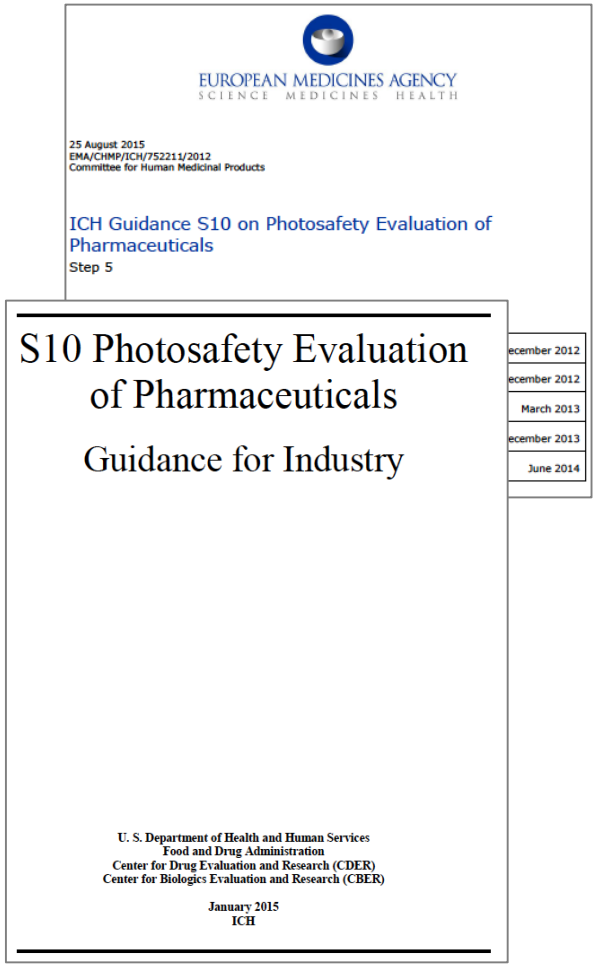
- No sufficiently developed in vitro models
- Guinea Pig Maximisation Test with adjuvant (OECD 406)
- Buehler Test in the Guinea Pig without adjuvant (OECD 406)
- Mouse Local Lymph Node Assay (OECD 429)
- Mouse Ear Swelling Test



Phototoxicity

PRECLINICAL SCIENCE

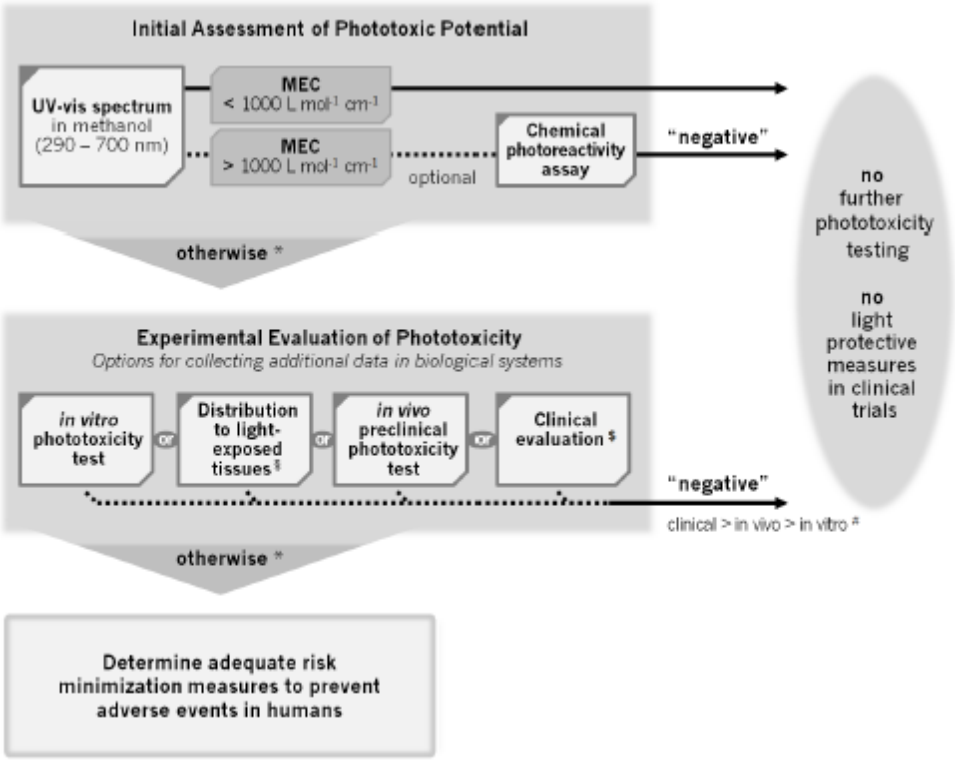
- Photoirritation
  - An acute light-induced tissue response to a photoreactive chemical
- Photoallergy
  - An immunologically mediated reaction to a chemical, initiated by the formation of photoproducts (e.g. protein adducts) following a photochemical reaction
- For a chemical to demonstrate phototoxicity and/or photoallergy, the following characteristics are critical:
  - absorbs light within the range of natural sunlight (290-700 nm)
  - generates a reactive species following absorption of UV-visible light
  - distributes sufficiently to light-exposed tissues (e.g., skin, eye) - default for topical products

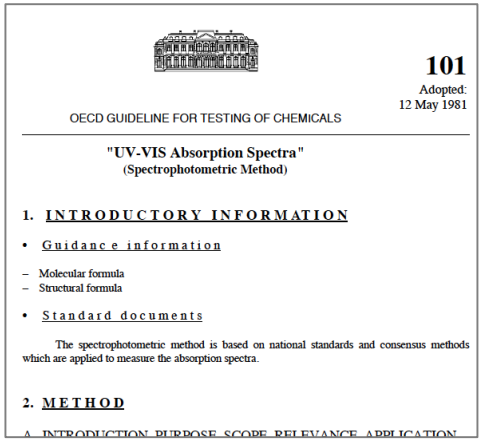


Phototoxicity testing

PRECLINICAL SCIENCE

- UV absorption





101
Adopted:
12 May 1981

OECD GUIDELINE FOR TESTING OF CHEMICALS

"UV-VIS Absorption Spectra"
(Spectrophotometric Method)

1. INTRODUCTORY INFORMATION

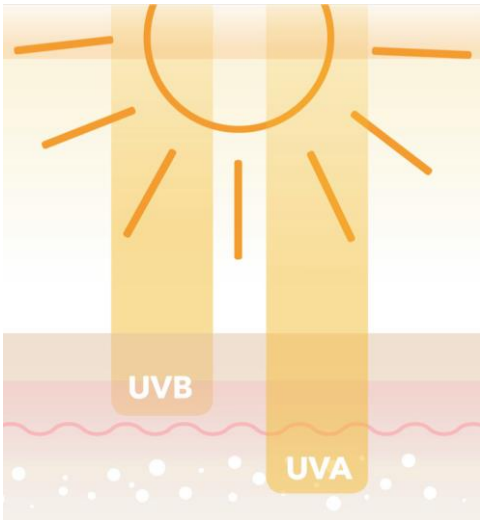
- Guidance information

– Molecular formula
– Structural formula

- Standard documents

The spectrophotometric method is based on national standards and consensus methods which are applied to measure the absorption spectra.

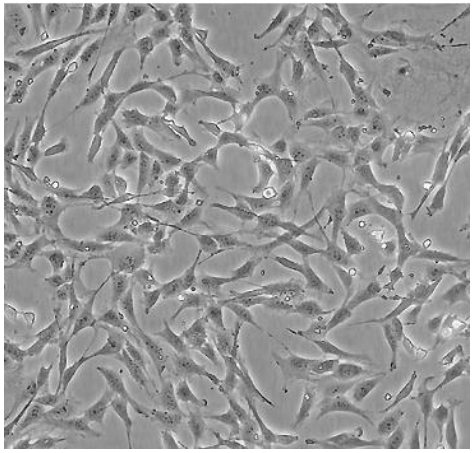
2. METHOD



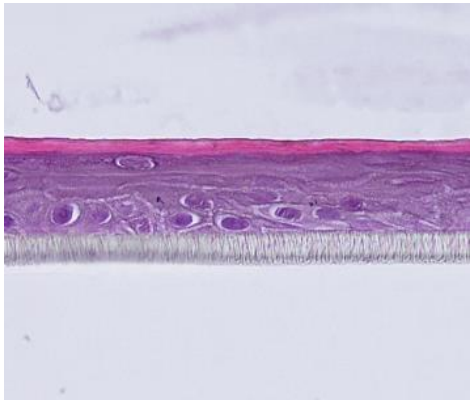
Phototoxicity testing

PRECLINICAL SCIENCE

- in vitro 3T3 NRU phototoxicity test (OECD 432)
  - BALB/c mouse 3T3 fibroblasts
  - Validated by ECVAM
  - 93% sensitivity, (84% specificity)
  - Water soluble compounds
  - 3T3 cytotoxicity in presence/ absence of UVA

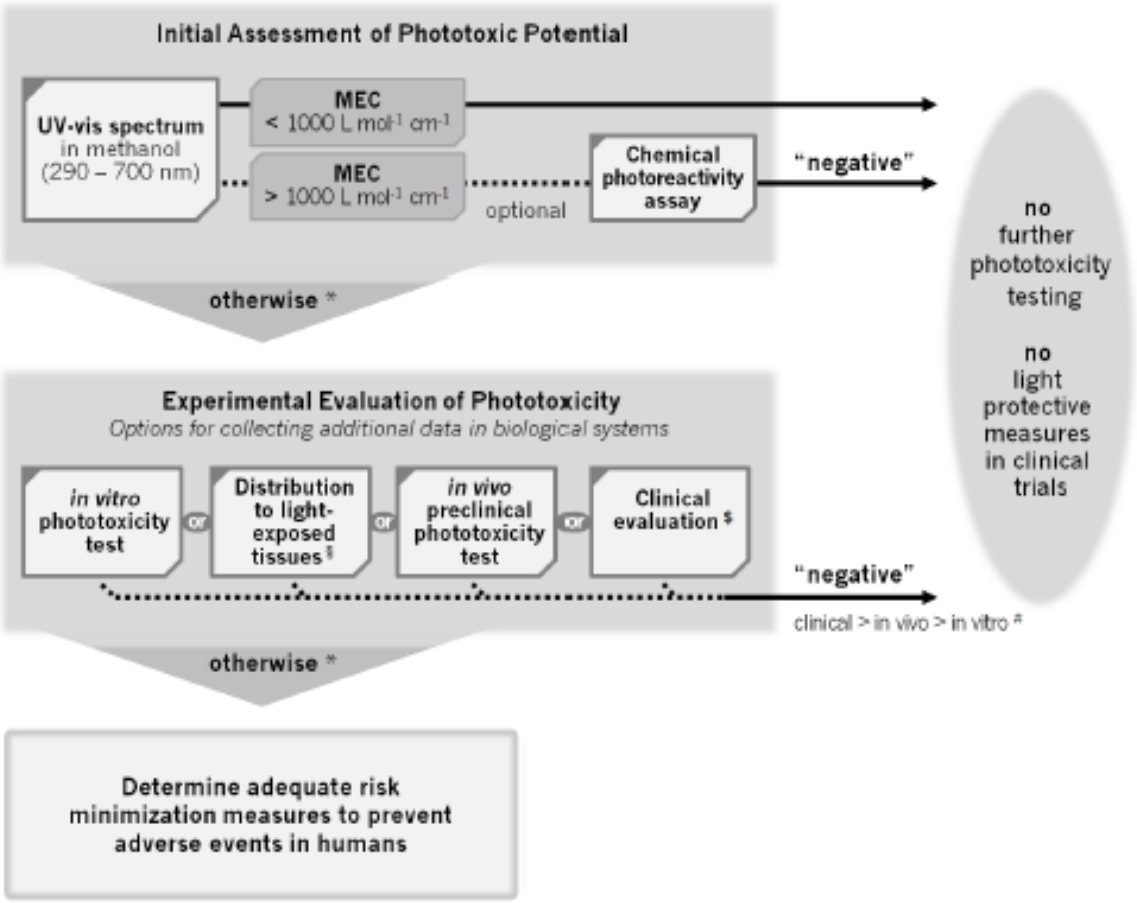


- In vitro reconstructed human skin models
  - Cytotoxicity measured by MTT assay



Phototoxicity testing

PRECLINICAL SCIENCE



Photocarcinogenicity

PRECLINICAL SCIENCE

- UV radiation is a known carcinogen in humans
  - Basal cell carcinoma
  - Squamous cell carcinoma
  - Malignant melanoma
- Pharmaceuticals may enhance the carcinogenicity of UVR
  - Shorten the time of onset to skin cancer development after a specific UVR dose
- Examples with FDA black box warning originating from in vivo animal studies:
  - Calcineurin inhibitors (tacrolimus, pimecrolimus)
  - Conflicting photocarcinogenicity data from in vitro and **animal studies**

Dermatology Reports 2010; volume 2:e13

Photocarcinogenicity of selected topically applied dermatological drugs: calcineurin inhibitors, corticosteroids, and vitamin D analogs

Catharina M. Lerche, Hans Christian Wulf

Department of Dermatology, Copenhagen University Hospital, Bispebjerg, Copenhagen, Denmark

Photocarcinogenicity

PRECLINICAL SCIENCE

- Hairless mice exhibit a dose-dependent response to daily whole-body exposure to fluorescent sunlamps
- As the daily dose increases, the mean latent period to tumor development decreases and tumor multiplicity increases
- Significant effects of
  - Cumulative dose distribution

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THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 77:139–143, 1981

Vol. 77, No. 1
Printed in U.S.A.

Photocarcinogenesis: An overview

PAUL DONALD FORBES, PH.D.
Center for Photobiology, Temple University Health Sciences Center, Philadelphia, Pennsylvania, U.S.A.

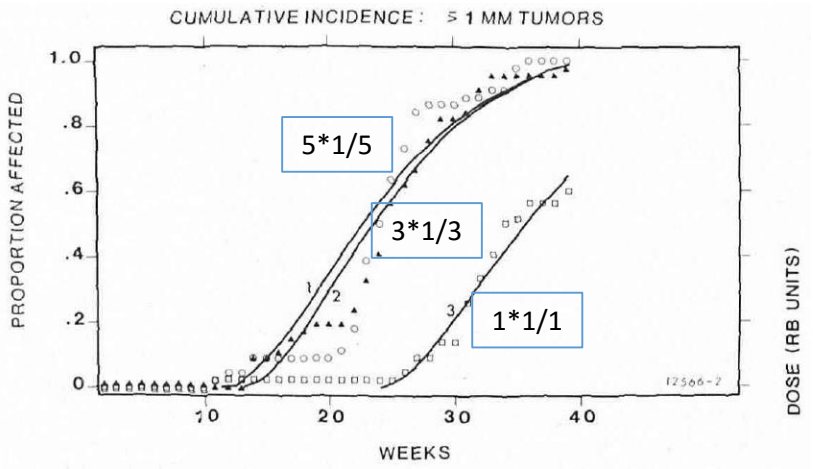

FIG 1. Tumor incidence (affected/survivors) for 3 groups of hairless mice receiving identical weekly dose of UVR (Westinghouse FS sunlamps). The weekly dose was given on 1 day (group 3), or one-third on each of 3 days (group 2) or one-fifth on each of 5 days (group 1). Group 3 had a significantly longer latent period than did the other 2 groups.

Photocarcinogenicity

PRECLINICAL SCIENCE

- Hairless mice exhibit a dose-dependent response to daily whole-body exposure to fluorescent sunlamps
- As the daily dose increases, the mean latent period to tumor development decreases and tumor multiplicity increases
- Significant effects of
  - Cumulative dose distribution
  - Strain (genetic background)

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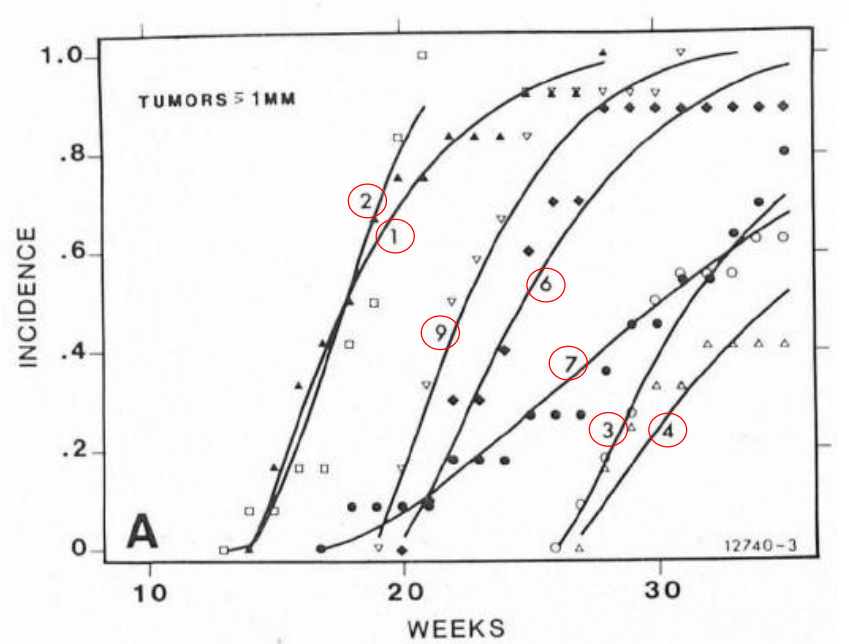


TABLE II. *Characteristics of test animals*

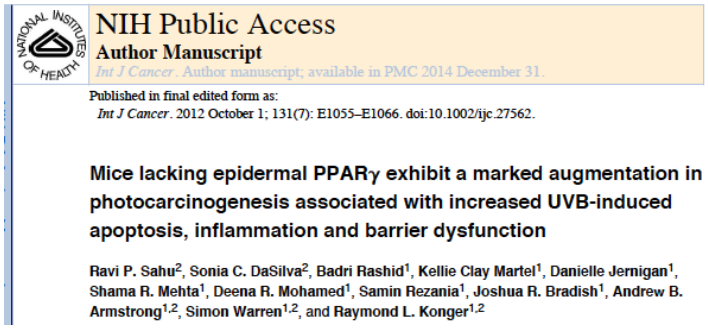
Stock or strain	Designation	Relevant genetics[a] Hair growth	Color	Source	Response curve # (Fig 3)
Stock	Skh:HR (type 1)	hr/hr	c/c	S&C Temple Univ.	1
	Skh:HR (type 2)	hr/hr	+/c, +/b, +/a	S&C Temple Univ.	9
Strain (F18)	HRA/Skh	hr/hr	c/c	S&C Temple Univ.	2
Stock	Skh:CRH	+/crh	+/c	S&C Temple Univ.	3
Strain (N8, F3)	C3H/HeN-hr	+/hr		NIH	6
Strain (F80)	HR/De/Hflcr	+/hr	p/p, b/b	Inst. Cancer Res.	5
Strain (F54)	HRS/J	+/hr	c/c	Jackson Lab.	7
Stock	Argonne hairless	+/hr	c/c	Argonne Nat. Lab.	8
Strain (F81+37)	BALB/cSkh-ab	+/ab	c/c, b/b	Univ. of California, Berkeley to S&C Temple Univ.	4

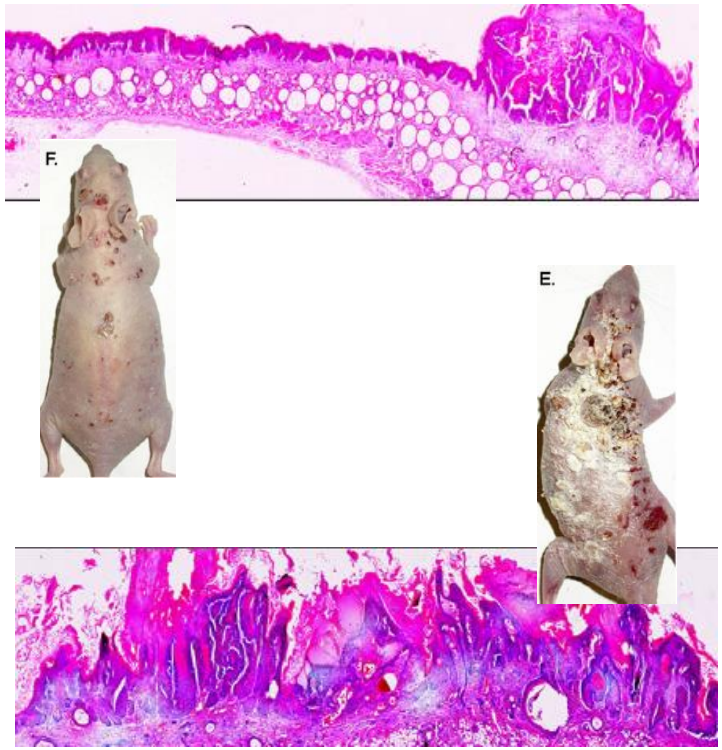
[a] Genotype symbols, c-albino, ab-asebia, b-brown, a-non agouti, crh-crytpothrix, hr-hairless, p-pink eyed.

Photocarcinogenicity

PRECLINICAL SCIENCE

- NTP technical report on the photocarcinogenesis study of aloe vera in SKH-1 mice (NTP TR 553)
- SKH-1 (*hr*⁻/*hr*⁻) mice
- 36 males/ 36 females
- Daily topic administration for 40 weeks (75µl)
  - Vehicle, 2 or 3 dose levels
- Daily exposure to simulated solar light (SSL)
  - minimal erythema dose (MED):minimal amount of radiation that causes slight erythema within 24 hours
- Animal survival analysis
- 12 weeks recovery
- In-life assessment of skin lesion onset and incidence
- Histopathology of skin
  - Squamous hyperplasia
  - Focal atypical squamous hyperplasia
- Histopathology tumor incidence
  - Squamous cell papilloma
  - Squamous cell carcinoma in situ
  - Squamous cell carcinoma

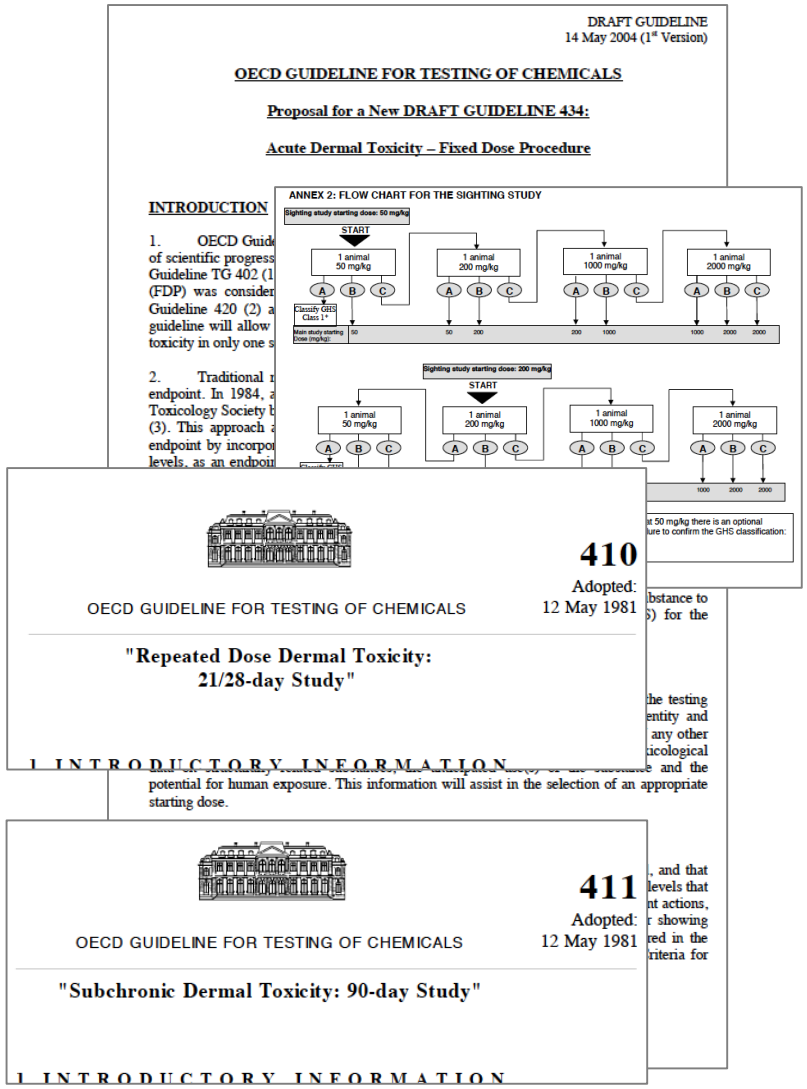




Dermal toxicity studies

PRECLINICAL SCIENCE

- Acute toxicity: OECD 434
  - Stepwise procedure
  - Rat, rabbit or guinea pig
  - One sex, 5 animals/group
  - Topical application (approx. 10% surface area)
  - Gauze dressing
  - 24 hrs exposure
  - 14 day observation period
  - Clinical observations, body weight, gross pathology, (histopathology)
- OECD 410 (21/28 day)
- OECD 411 (90 days)
  - Rat, rabbit, guinea pig, **minipigs**
  - Mortality, clinical observations (administration site), food intake, body weight, clinical pathology, gross pathology, histopathology



DRAFT GUIDELINE
14 May 2004 (1st Version)

OECD GUIDELINE FOR TESTING OF CHEMICALS

Proposal for a New DRAFT GUIDELINE 434:

Acute Dermal Toxicity – Fixed Dose Procedure

INTRODUCTION

ANNEX 2: FLOW CHART FOR THE SIGHTING STUDY

410
Adopted:
12 May 1981

OECD GUIDELINE FOR TESTING OF CHEMICALS

"Repeated Dose Dermal Toxicity:
21/28-day Study"

1 INTRODUCTORY INFORMATION

411
Adopted:
12 May 1981

OECD GUIDELINE FOR TESTING OF CHEMICALS

"Subchronic Dermal Toxicity: 90-day Study"

1 INTRODUCTORY INFORMATION

Repeat dose dermal toxicity

PRECLINICAL SCIENCE

- Example: CLOBEX Spray
  - 0.05% clobetasol propionate
  - Plaque psoriasis
- 3 month dermal toxicity study in Sprague Dawley rats with a 4 week recovery
- 90 day dermal toxicity study in Yucatan pigs
- 9 month dermal toxicity study with a 1 month recovery in Hanford minipigs

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:
21-835

PHARMACOLOGY REVIEW

Minipigs in dermal toxicity testing

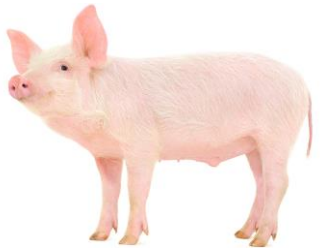
PRECLINICAL SCIENCE

- Pig is accepted as appropriate species for topically applied products
- Minipigs preferred over domestic pigs because of small size, easier handling, extensive background information
- Several strains of minipigs available (Sinclair, Yucatan, Hanford, Göttingen, etc.)
- Göttingen strain: pale skin, slow growth rate, extensive historical data

Sinclair

Yucatan

Hanford



Göttingen

http://www.sinclairbioresources.com

Comparative histology

PRECLINICAL SCIENCE

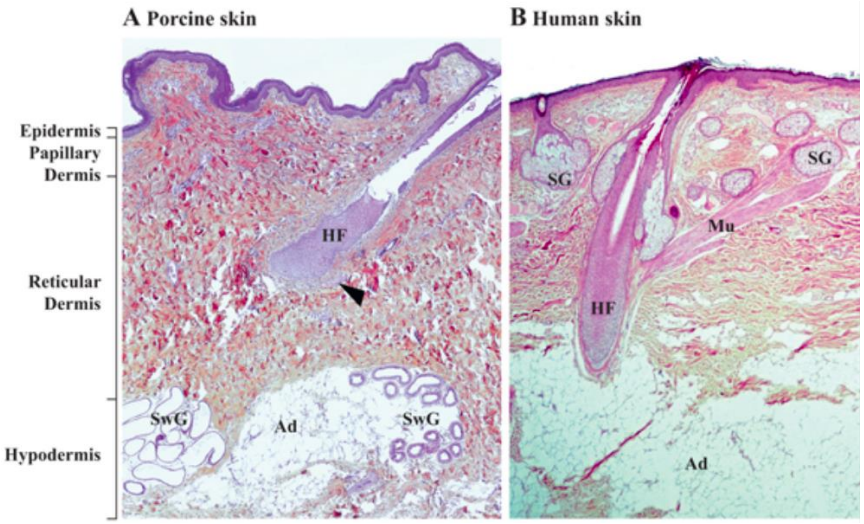
High similarity, except for

- Thicker stratum corneum
- Presence of interfollicular muscle
- Extensive apocrine glands do not contribute to thermoregulation
- Eccrine sweat glands limited to snout and carpus

Eur J Dermatol. 2013 Jul-Aug;23(4):456-66. doi: 10.1684/ejd.2013.2060.

Comparative histology and immunohistochemistry of porcine versus human skin.

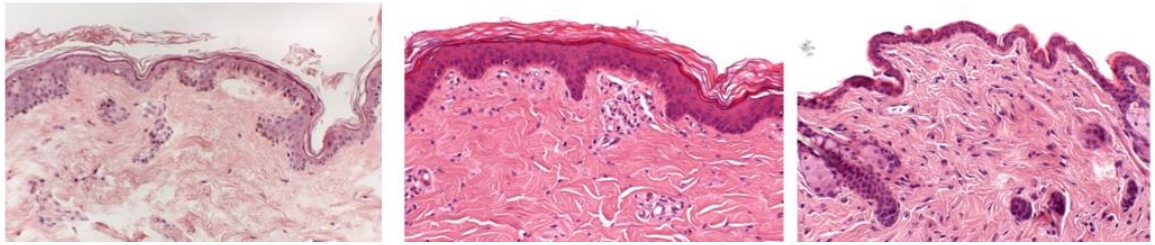
Debeer S[1], Le Luduec JB, Kaiserlian D, Laurent P, Nicolas JF, Dubois B, Kanitakis J.



Species comparison

PRECLINICAL SCIENCE

HUMAN	PIG	MOUSE
Firmly attached to underlying structures	Firmly attached to underlying structures	Loosely attached to underlying structures
Limited hair cover	Limited hair cover	Hairy
4-5 cell layers of the epidermis (abdominal area)	5-6 cell layers of the epidermis (abdominal area)	1-2 cell layers of the epidermis (abdominal area)
Presence of rete ridge structure	Presence of rete ridge structure	Absence of rete ridge structure
Well-vascularised dermis	Well-vascularised dermis	Limited vascularisation
Selectively-permeable	Selectively-permeable	Permeable



hair follicles/cm^2
(Stricker-Krongrad et al. 2016)

11 ± 1

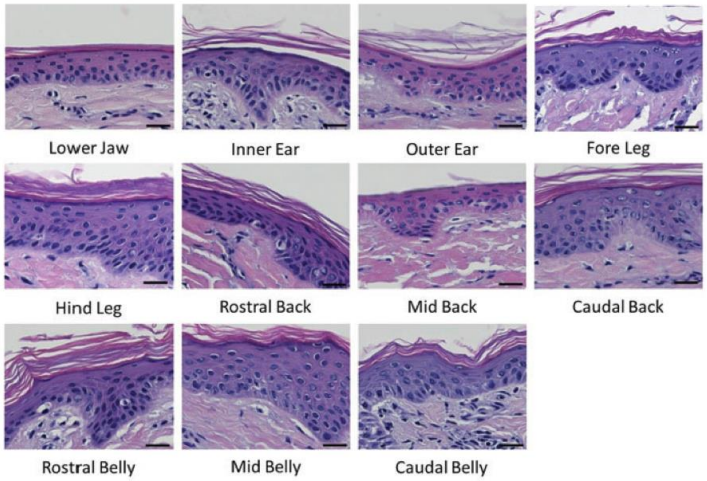
11 ± 1

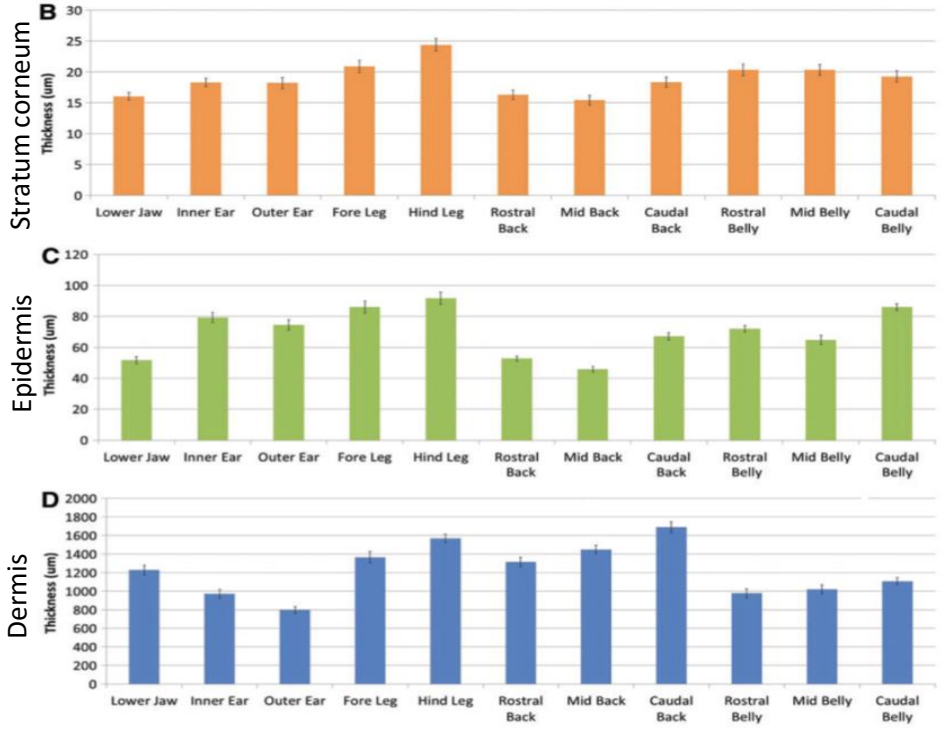
658 ± 38
(hairless mouse: 75 ± 6)

Regional variation

PRECLINICAL SCIENCE

- Thickest epidermis/ stratum corneum in hind leg
- Thinnest epidermis/ stratum corneum in mid back
- Thickest dermis in caudal back
- Thinnest dermis in outer ear



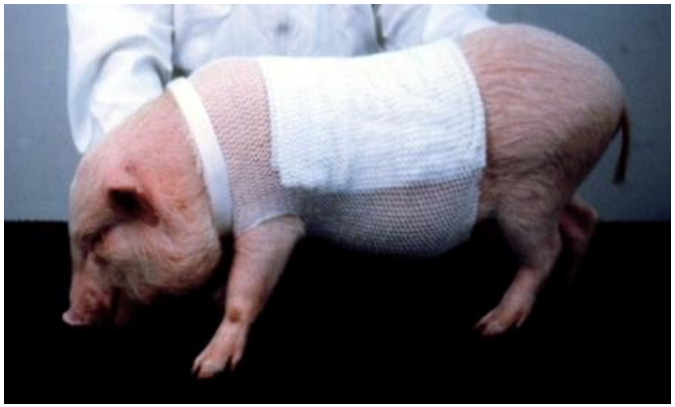


Turner NJ, Pezzone D, Badylak SF. Regional variations in the histology of porcine skin. Tissue Eng Part C Methods. **2015** Apr;21(4):373-84

Dermal toxicity studies in Göttingen minipigs

PRECLINICAL SCIENCE

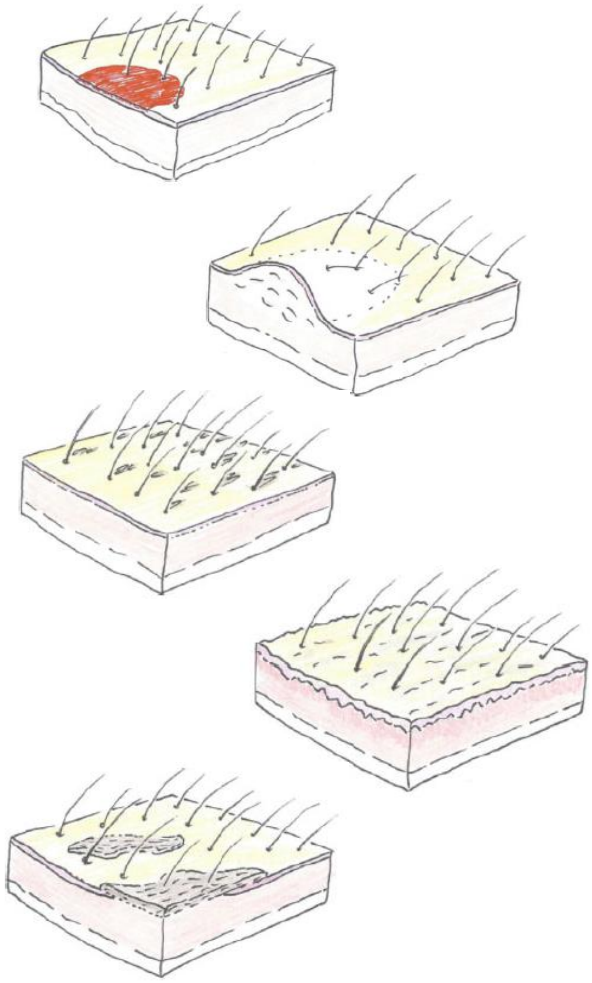
- Preparation of dosing site
  - Tape stripping to allow penetration/absorption
  - Marking the dose site by tattooing
- Test article application
  - Evenly applied to dorsal skin (approx. 10%)
  - Occlusion with wound dressing
  - After dosing time, dressing is removed and dosing site is washed
- Challenges
  - Potential for cross-contamination by rubbing against the pen
    - Separate housing for control animals
    - Strict separation of equipment and clothing
  - Collections of electrocardiograms is challenging
  - Labor intensive / training of personnel
  - Severe erythema, erosions, ulcers, abscesses, and necrosis are considered painful
    - Administration of systemic analgesics
    - Discontinue dosing



Macroscopic observations

PRECLINICAL SCIENCE

- Erythema
- Edema
- Atonia
- Desquamation
- Fissuring
- Scab formation
- Exfoliation



Original Article

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2016, Vol. 44(3) 382-390

DOI: 10.1177/0192623315622585
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Dermatotoxicology: Safety Evaluation of Topical Products in Minipigs: Study Designs and Practical Considerations

Cynthia Willard-Mack[1], Thulasi Ramani[1], and Carol Auletta[1]

Erythema	Edema
0—None	0—None
1—Slight	1—Slight
2—Moderate	2—Moderate
3—Severe	3—Severe

Other:

- Atonia—A decrease in normal elasticity (resilience) of the skin
  - 1—Slight (slight impairment of elasticity)
  - 2—Moderate (slow return to normal)
  - 3—Marked (no elasticity)
- Desquamation—Scaling/flaking of the epidermis
  - 1—Slight (slight scaling)
  - 2—Moderate (scabs and flakes)
  - 3—Marked (pronounced flaking with denuded areas)
- Fissuring—Cracks in the skin
  - 1—Slight (definite cracks in epidermis)
  - 2—Moderate (cracks in dermis)
  - 3—Marked (cracks and bleeding)
- Scab formation
  - F—Focus/foci present
  - P—Patches present
- Exfoliation—Sloughing of scabs/eschar tissue
  - P—Present
- Tissue Damage/Necrosis—Dead skin; blanched or blackened dead tissue

Microscopic observations

PRECLINICAL SCIENCE

- INHAND manuscripts

Pathol 2013; 26 (3 Suppl): 27S–57S

Proliferative and Non-Proliferative Lesions of the Rat and Mouse Integument

Lars Mecklenburg[1], Donna Kusewitt[2], Carine Kolly[3], Silke Treumann[4], E. Terence Adams[5], Kelly Diegel[6], Jyoti Yamate[7], Wolfgang Kaufmann[8], Susanne Müller[9], Dimitry Danilenko[10], and Alys Bradley[11]

[1] *mecklenburg-consulting, Hamburg, Germany*
[2] *UT MD Anderson Cancer Center, Smithville, Texas, USA*
[3] *Novartis Pharma AG, Basel, Switzerland*
[4] *BASF SE, Ludwigshafen, Germany*
[5] *Experimental Pathology Laboratories, Inc, Research Triangle Park, North Carolina, USA*
[6] *Hoffmann La-Roche Nonclinical Safety, Nutley, New Jersey, USA*
[7] *Osaka Prefecture University, Osaka, Japan*
[8] *Merck Serono Research & Development, Darmstadt, Germany*
[9] *Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany*
[10] *Genentech, South San Francisco, California, USA*
[11] *Charles River, Tranent, Edinburgh, UK*

Abstract

The INHAND (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) project is a jo
tiative of the societies of toxicological pathology from Europe (ESTP), Great Britain (BSTP), Japan (JSTP) and North America (STP)
n is to develop an internationally-accepted nomenclature for proliferative and non-proliferative lesions in laboratory rodents. A wid
cepted international harmonization of nomenclature in laboratory animals will decrease confusion among regulatory and scient
search organizations in different countries and will provide a common language to increase and enrich international exchanges of
rmation among toxicologists and pathologists. The purpose of this publication is to provide a standardized nomenclature for classify
croscopical lesions observed in the integument of laboratory rats and mice. Example colour images are provided for most lesions. T
ndardized nomenclature presented in this document and additional colour images are also available electronically at http://www.gore
g. The nomenclature presented herein is based on histopathology databases from government, academia, and industrial laborator
roughout the world, and covers lesions that develop spontaneously as well as those induced by exposure to various test materials. (D
.1293/tox.26.27S; J Toxicol Pathol 2013; 26: 27S–57S)

Keywords: diagnostic pathology; histopathology; nomenclature; rodent pathology; integumentary system; skin; rodent.

cial disclosure: No money was paid for the preparation of this
pt. During preparation of this manuscript, salaries of contribu-
paid by their respective employer. None of the content of the
pt contains any information that could be patentable or claimed

Introduction

This manuscript provides a standardized nomencl

NON-PROLIFERATIVE TERMS	Common	Uncommon
Atrophy, adnexal		x
Atrophy, dermal		x
Atrophy, epidermal		x
Cyst, squamous		x
Edema, dermal	x	
Edema, intercellular, epidermal	x	
Edema, intracellular, epidermal	x	
Erosion/ulcer	x	
Hyperkeratosis, adnexal		x
Hyperkeratosis, epidermal	x	
Infiltrate, inflammatory cell, epidermal	x	
Inflammation, adnexal	x	
Abscess	x	
Necrosis, adnexal	x	
Necrosis, epidermal	x	
Pustule	x	
Vesicle	x	

PROLIFERATIVE TERMS	Common	Uncommon
Non-Neoplastic		
Hyperplasia, adnexal		x
Hyperplasia, epidermal	x	
Hyperplasia, melanocyte		x
Neoplastic		
Melanoma, benign	x*	
Melanoma, malignant	x*	

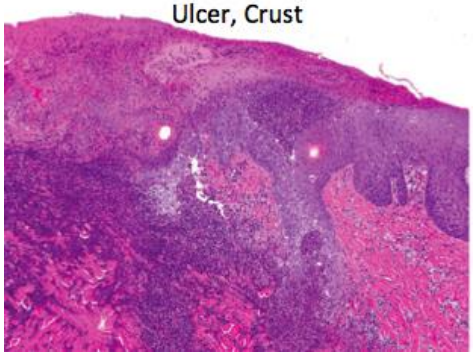
*melanomas described in Sinclair and MeLiM aka Libechov strains; but not described in white skinned minipigs such as Göttingen strain

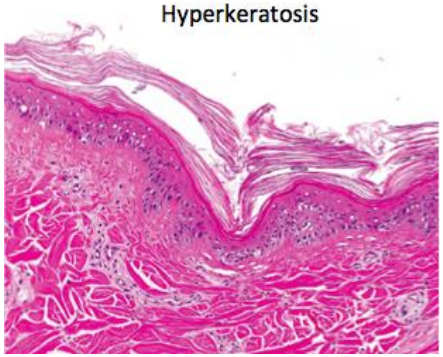
Microscopic observations

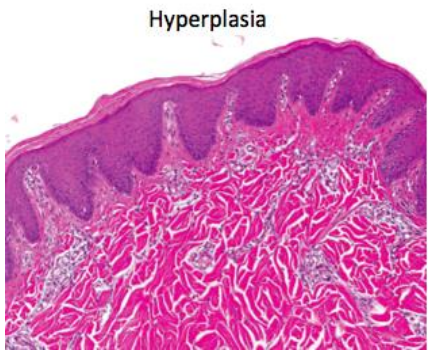
PRECLINICAL SCIENCE

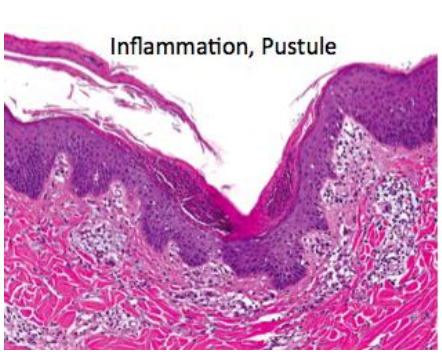
- Background data from literature
  - Stricker-Krongrad et al. 2016

Breed	Findings	Male	Female
Göttingen		*n* = 143	*n* = 143
	Crust, focal, minimal to slight	4.2%	4.9%
	Hyper/parakeratosis, focal to diffuse, minimal	2.1%	2.8%
	Epidermal/subepidermal edema, focal, minimal to slight	1.4%	4.9%
	Mononuclear/inflammatory cells, focal, minimal to moderate	6.3%	7.0%
Yucatan		*n* = 18	*n* = 21
	Mononuclear infiltrates, focal, minimal	5.6%	0.0%
Hanford		*n* = 60	*n* = 59
	Acute inflammation, dermis, minimal	0.0%	1.7%
	Chronic inflammation, dermis, minimal	6.7%	5.1%
	Chronic inflammation, perifollicular, minimal	0.0%	1.7%
	Lymphohistiocytic inflammation, multifocal, mild	0.0%	1.7%









Documentation

PRECLINICAL SCIENCE

- Correlation with clinical observations
  - Terminology
  - Systematic diagnostic approach
  - Consistency
    - Clinical observations
    - Gross pathology
    - Histopathology

Session: Case Studies of Cutaneous Toxicity, Direct and Systemic

Recommended Diagnostic Approach to Documenting and Reporting Skin Findings of Nonhuman Primates from Regulatory Toxicity Studies

Toxicologic Pathology
2016, Vol. 44(4) 591-600

DOI: 10.1177/0192623316638445
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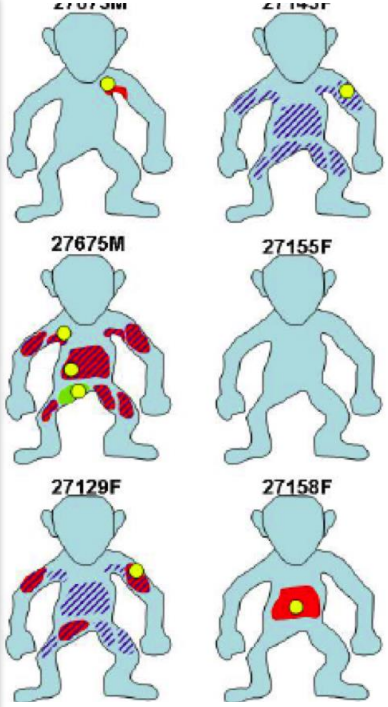
Lars Mecklenburg[1] and Annette Romeike[1]

```
                                Test Item (dosage)      1M
                                ---------- ----------- --
                                                mg/kg b.i.d. 0
-------------------------------------------------------------
      Phase: All
-------------------------------------------------------------
      Category                                      Group/S
          Observation                          Number in Gro
-------------------------------------------------------------
      Head
          squamous skin, face, slight
          reddened skin, face, slight
          reddened skin, head, slight
          discharge, both nasal wings, bloody
          discharge, both nasal wings, clear
          discharge, both nasal wings, crusted
          discharge, left nasal wing, bloody
          discharge, right nasal wing, clear
      Eye/s
          reddened skin, both eyes, slight
      Trunk
          squamous skin, both inguinal, slight
          squamous skin, chest, slight
          squamous skin, lower abdomen, slight
          squamous skin, upper abdomen, slight
          squamous skin, whole abdomen, slight
          reddened skin, both inguinal, severe
          reddened skin, both inguinal, slight
          reddened skin, chest, slight
          reddened skin, lower abdomen, slight
          reddened skin, whole abdomen, slight
-------------------------------------------------------------
```



Thank you !

PRECLINICAL SCIENCE

Föll, Mecklenburg & Partner GmbH

www.preclinical-science.com

Althausweg 158 | 48159 Münster | Germany

Phone: +49 (251) 265 26 41

eMail: office@preclinical-science.com

Dr. Roman Föll, Dr. Lars Mecklenburg,
Dr. Geertje Lewin