The skin as a metabolizing organ of pro-haptens

Understanding of the fate of reactive chemicals in the skin

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STUDIES ON THE SENSITIZATION OF ANIMALS WITH SIMPLE CHEMICAL COMPOUNDS*

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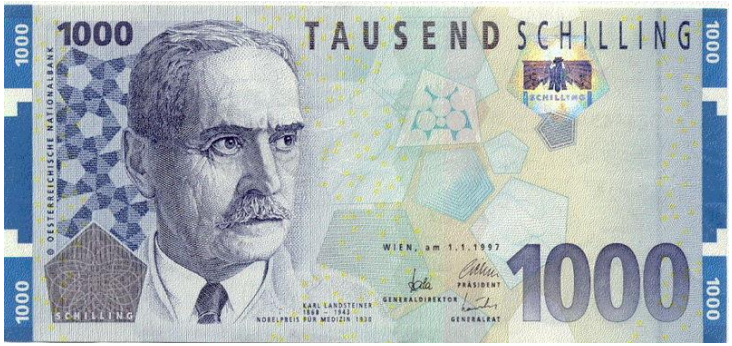
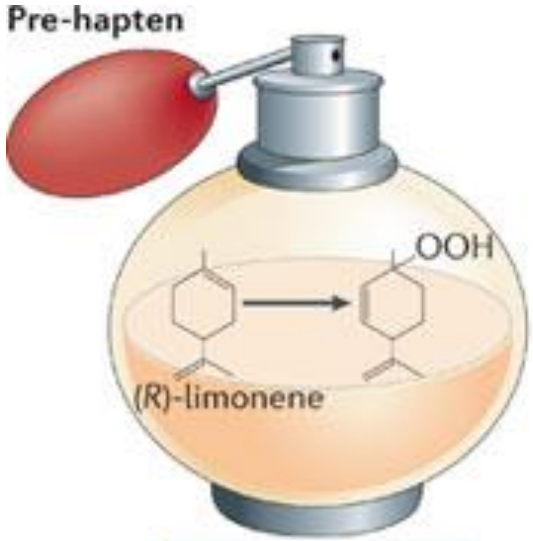
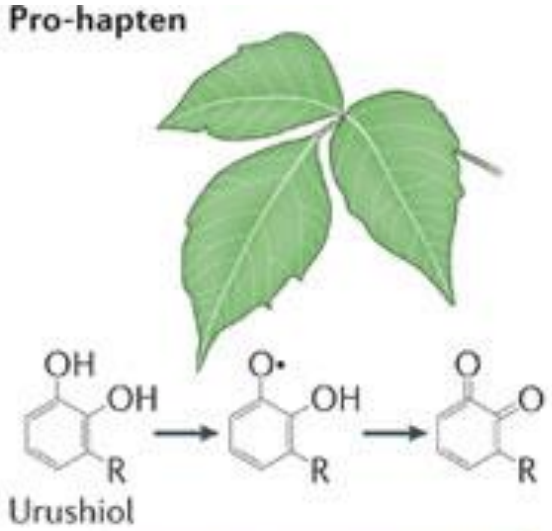
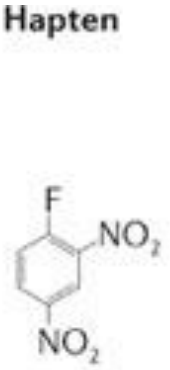
Plate 30

(Received for publication, January 25, 1935)









Pre-haptens oxidize before skin contact

Pro-haptens are oxidized by the host after contact

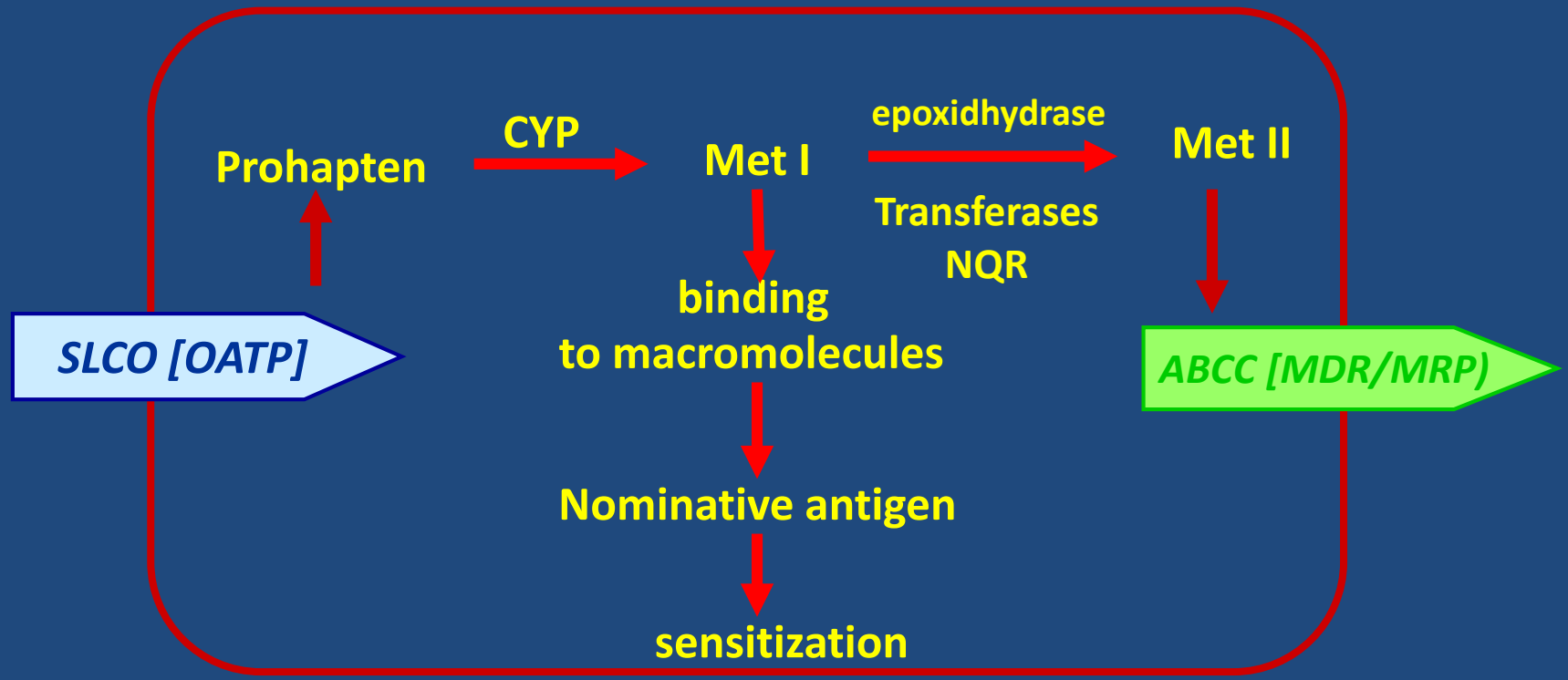
Complete haptens are directly reactive

DH Kaplan et al., Nat Rev Immunol 12 (2012) 114-124

The skin as a metabolizing organ of pro-haptens

Understanding of the fate of reactive chemicals in the skin

Phase I: CYP – Phase II: EH/ Transferases –
Phase III: Transporter proteins

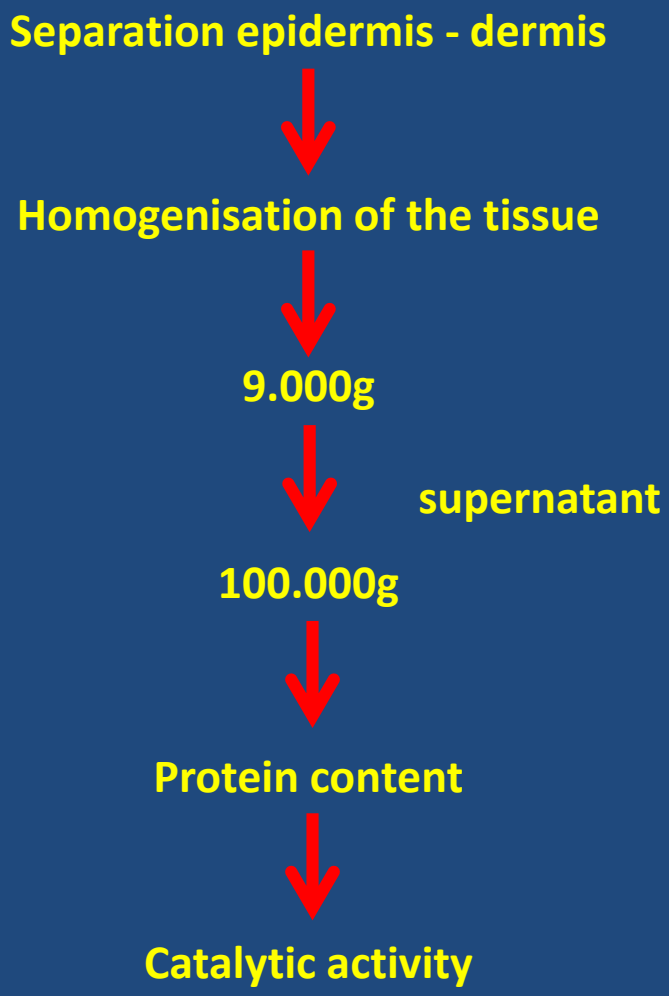


ABCC: ATP binding cassette C transporters (MRP/ MDR)
SLCO: solute carrier organic anion transporter (OATP)

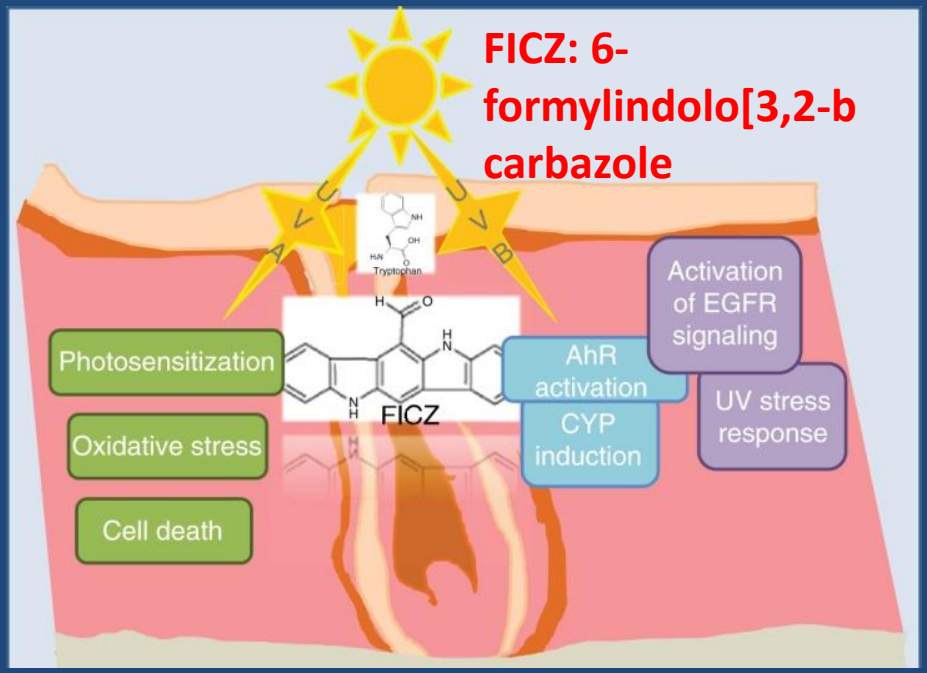
Xenobiotica metabolising enzymes in the skin

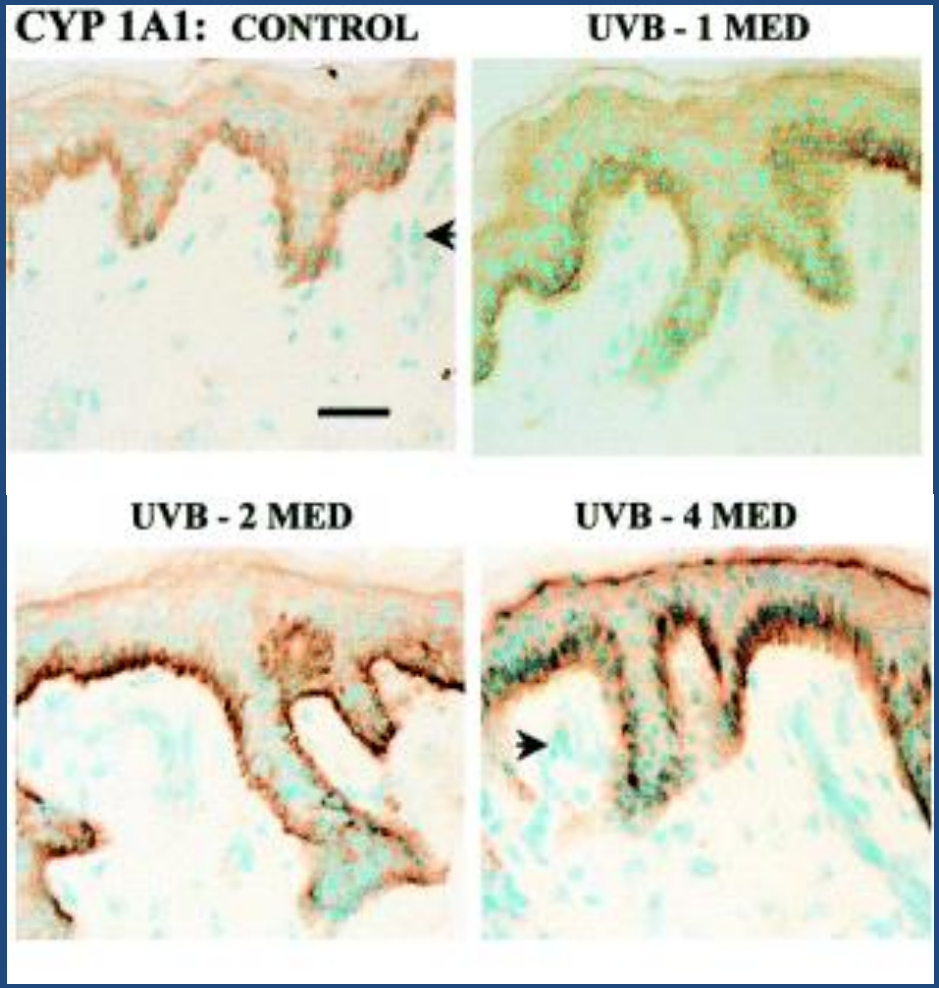
Cytochrome P450
Flavin-dependent monooxygenases (FMO
Cyclooxygenases (COX1/ COX2)
Alcohol dehydrogenase (ADH)
Aldehydedehydrogenase (ALDH)
Epoxide hydrolase (EH)
NAD(P)H:quinone reductase (NQR)
Glutathione S-transferase (GST)
UDP-glucuronyosyltransferase (UGT)
Sulfotransferase (SULT)
N-Acetyltransferase (NAT)
Esterase/ amidase

Skin microsomes



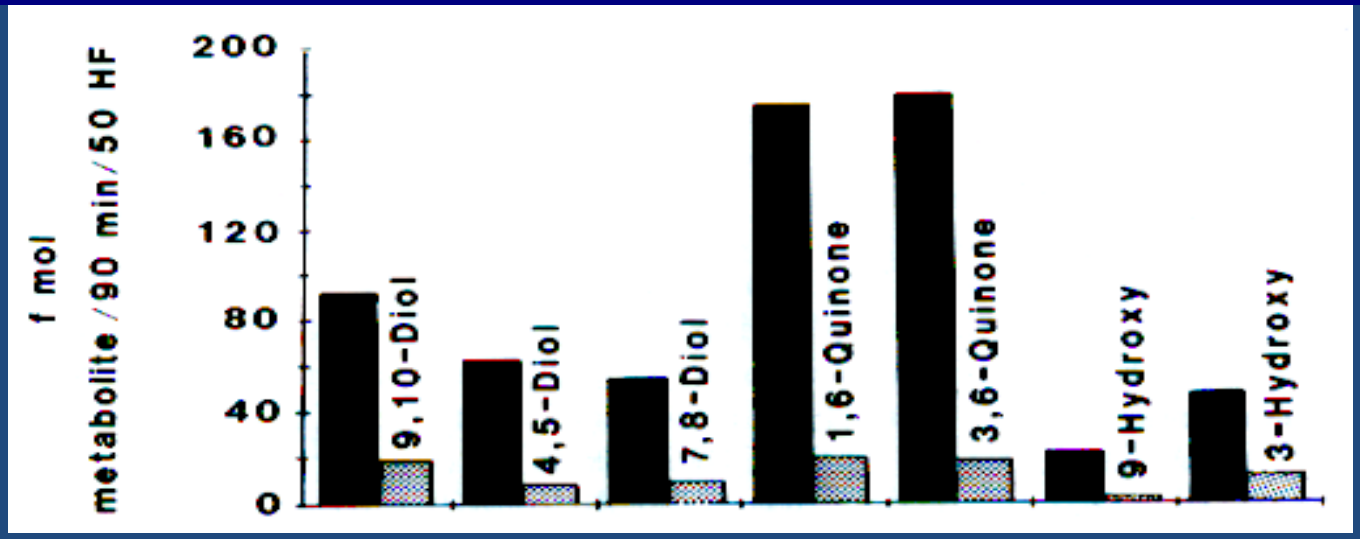
CYPs in human skin: induction by UVB

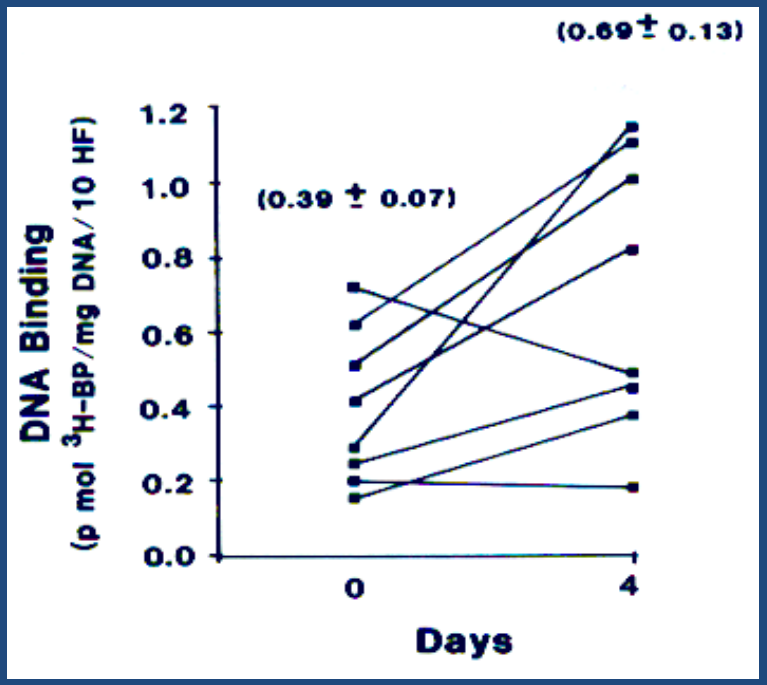


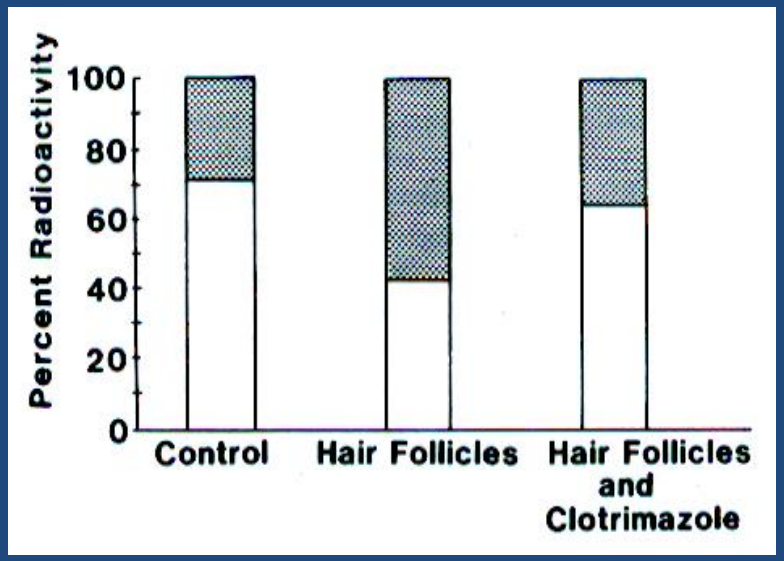


SK Katiyar et al., JID 114 (2000) 328-333; DN. Syed & H Mukhtar, JID (2015) 135, 1478–1481

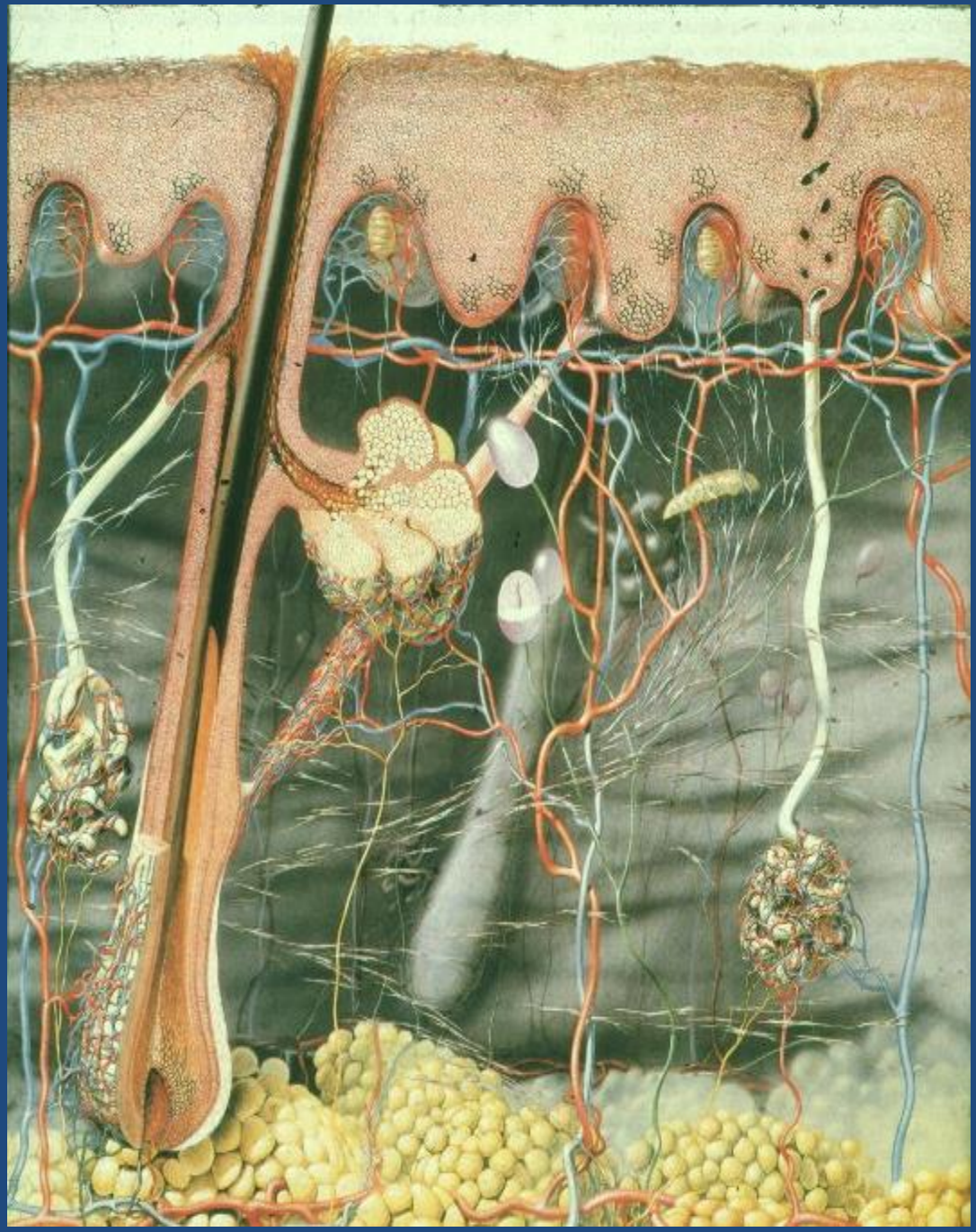
Inhibition of Benz(a)pyrene-Metabolism by Clotrimazole



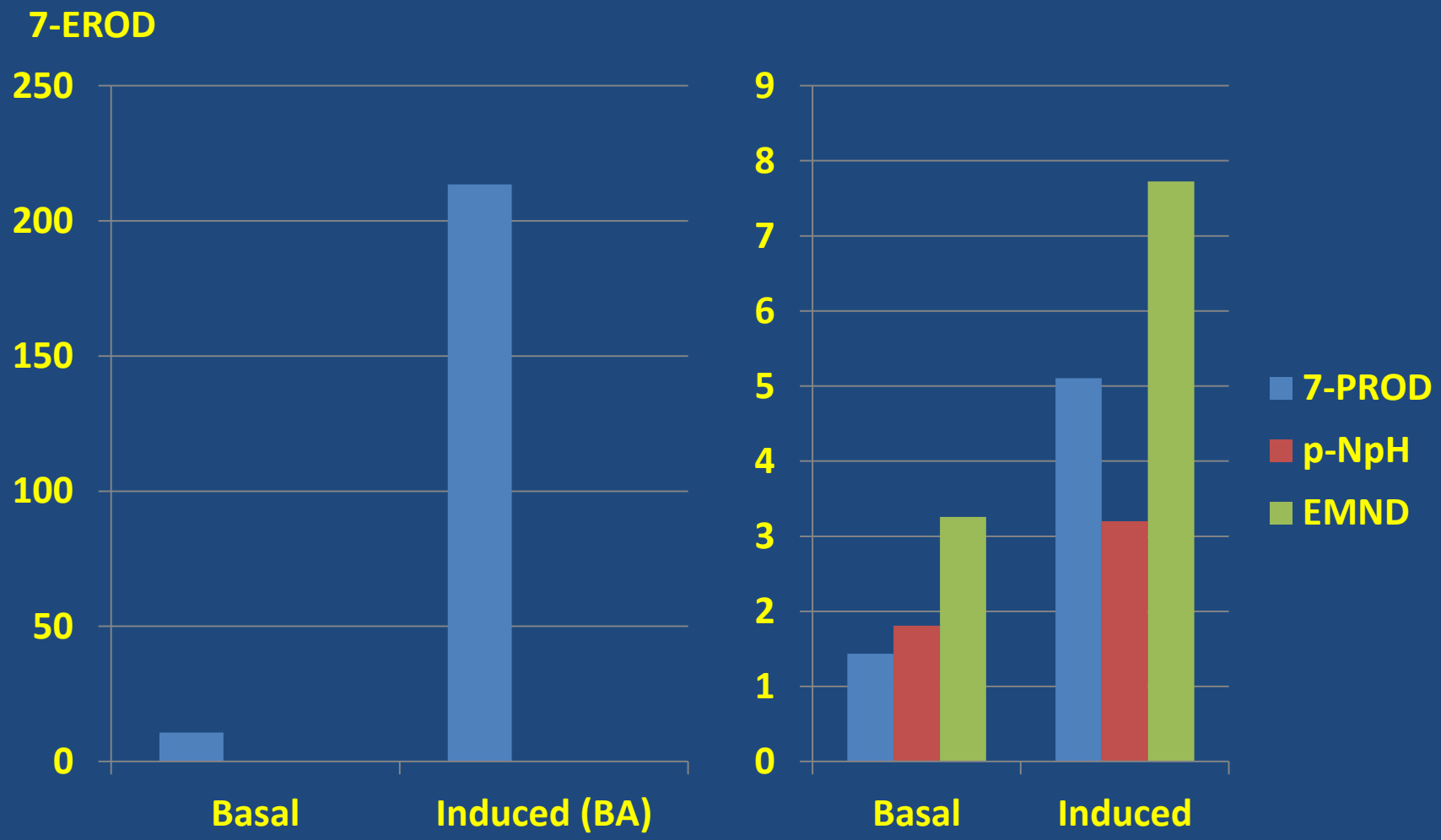




H.F.Merk et al., JID 1987



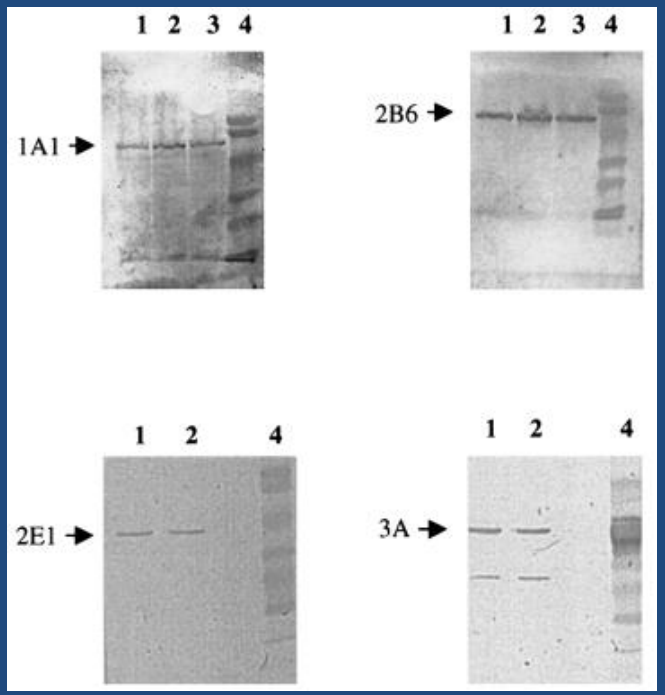
Multiple CYPs in human keratinocytes

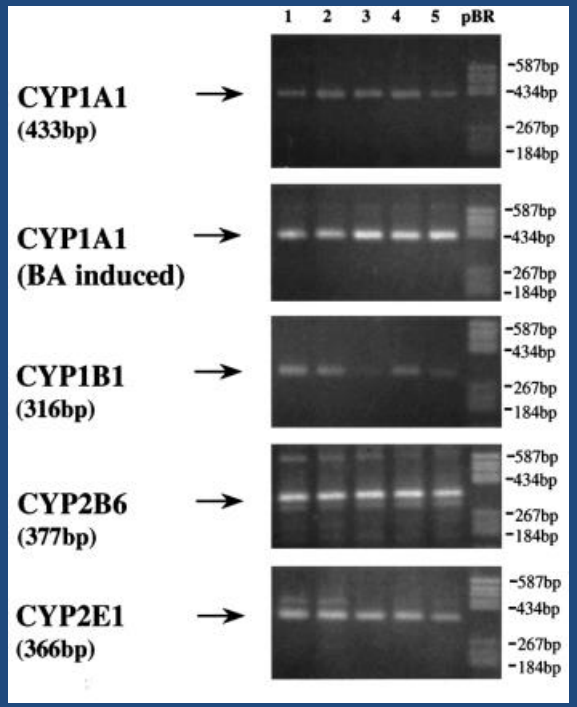


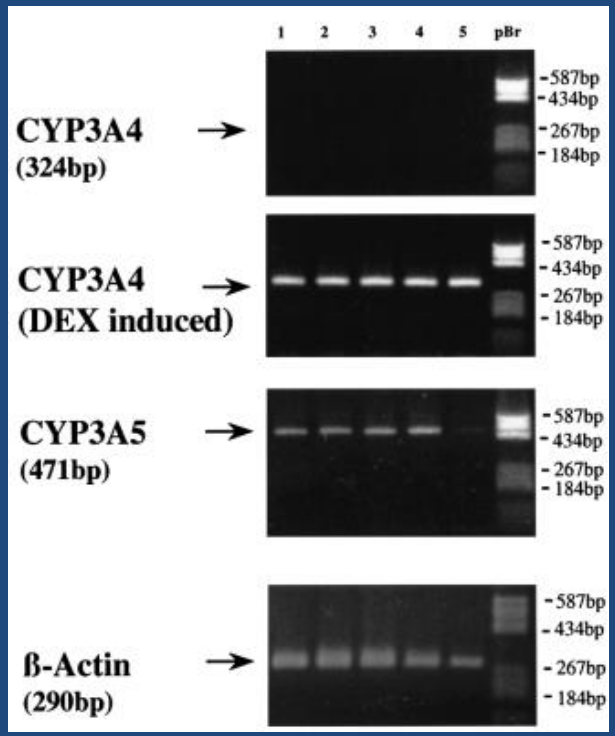
7-EROD (inducer Benzanthracene), 7-PROD (phenobarbital); p-NpH (ethanol); EMND (dexamethasone)

Baron et al., JID 116 (2001) 541-548

Multiple CYPs in human keratinocytes

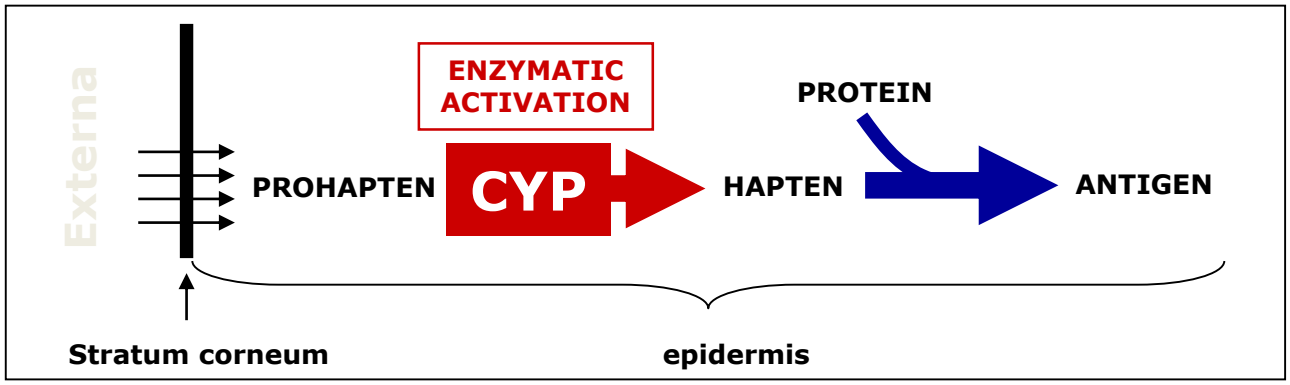






Baron et al., JID 116 (2001) 541-548

Activation of prohaptens is mediated by CYPs expressed in skin cells

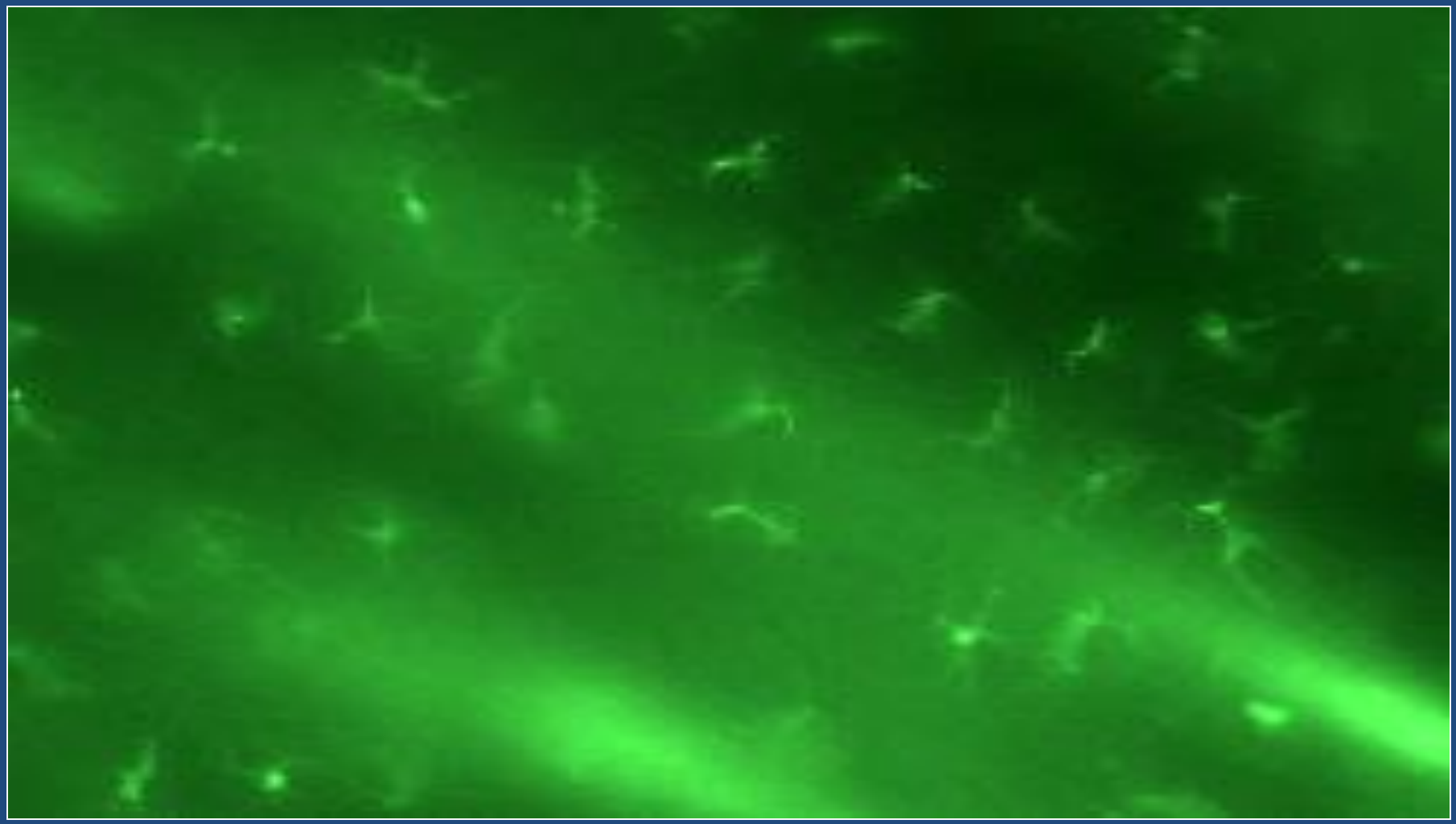


	CYP amount (pmol)
Skin specific rhCYP cocktail	
CYP1A1	3.6
CYP1B1	2.0
CYP2B6	0.035
CYP2E1	11
CYP3A5	5.6
total CYP amount	22

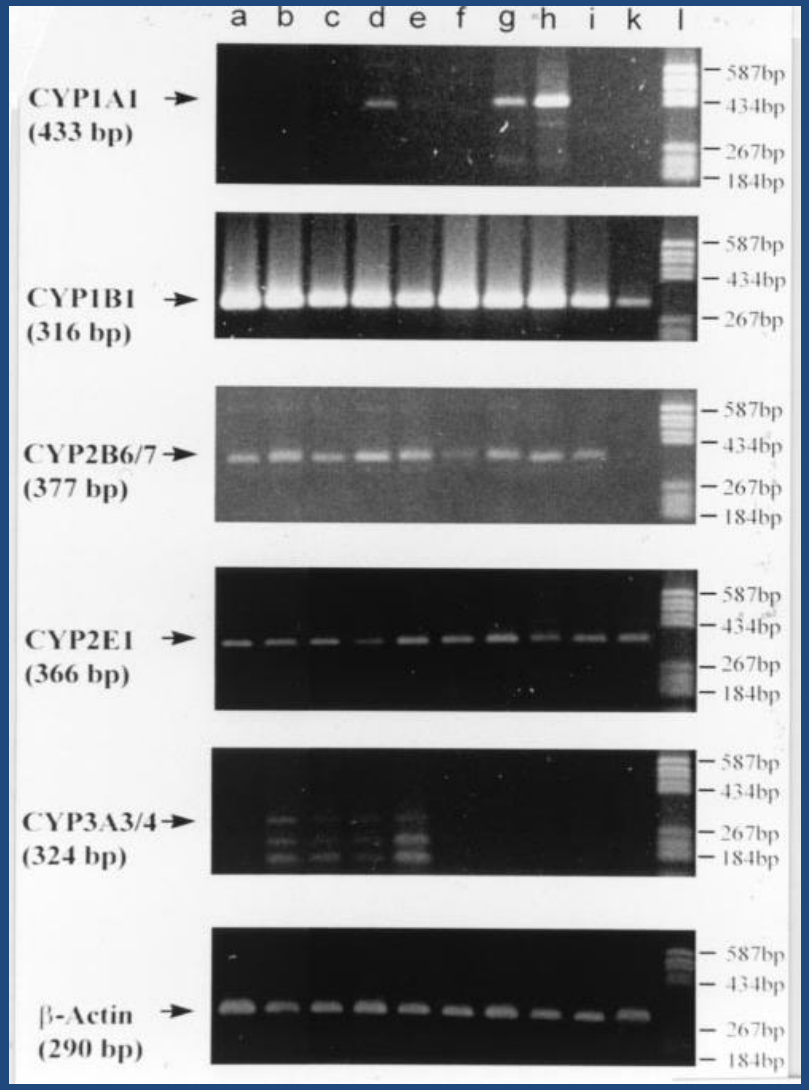
J Invest Dermatol, 127: 1145–1153, 2007

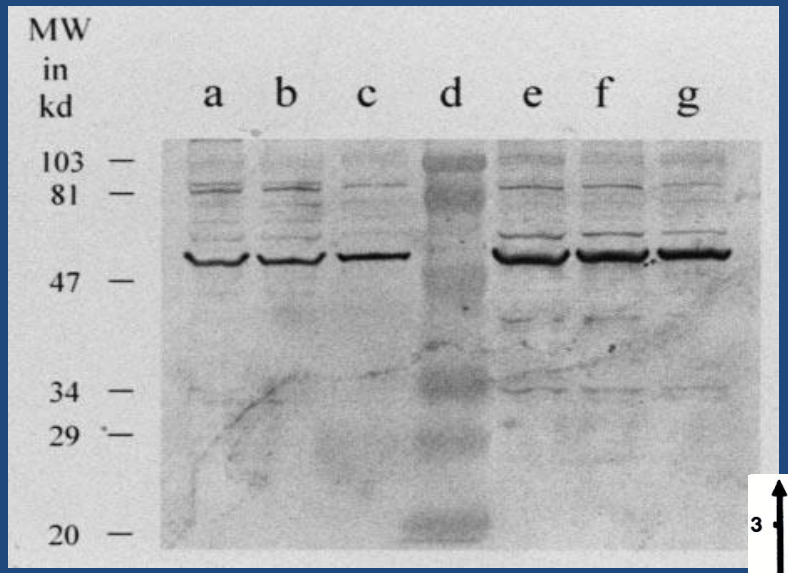
Langerhans-cells

Ia+ dendritic cells (murine skin / FITC stain)

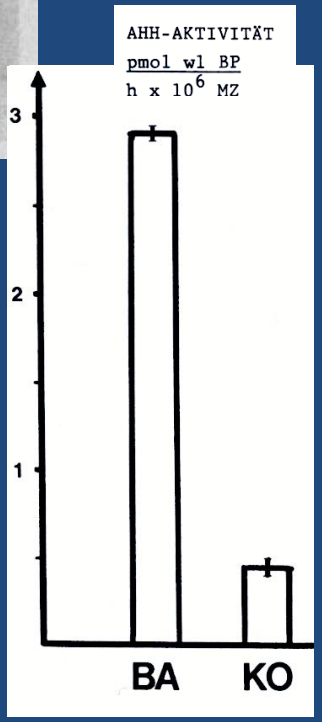


CYPs in monocytes

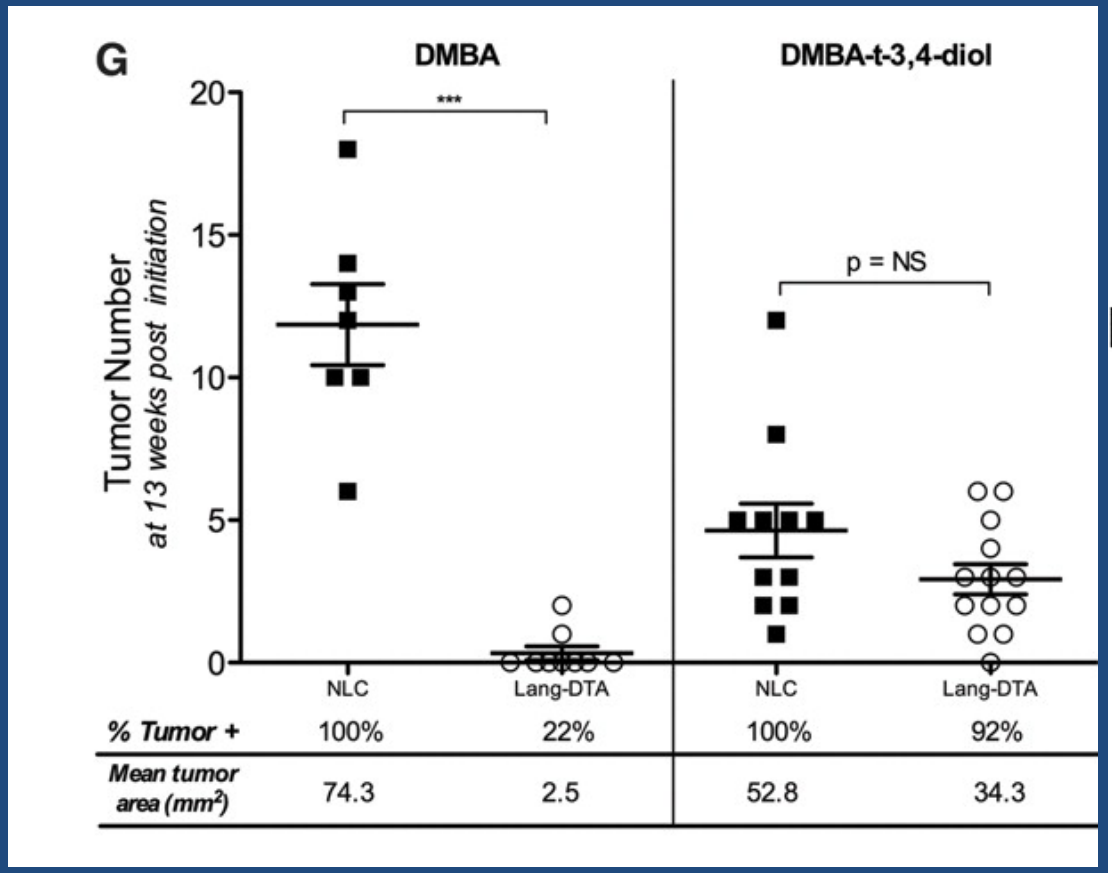


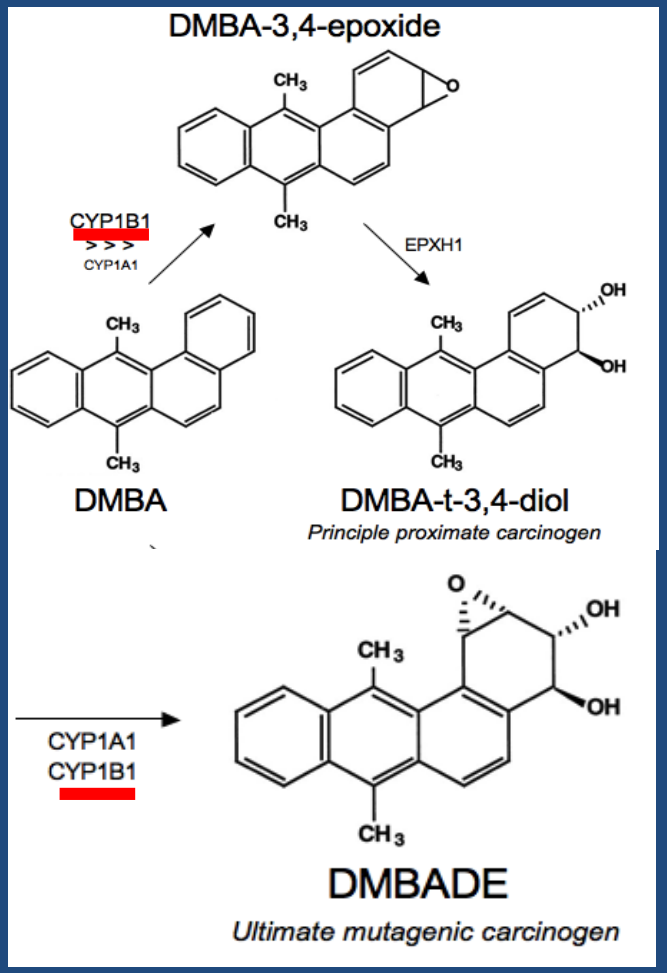


V Schenk, Thesis, 1988,
JM Baron et al., Biochem
Pharmacol 56 (1998) 1105-1110



Less tumor induction by DMBA in LC-deficient mice



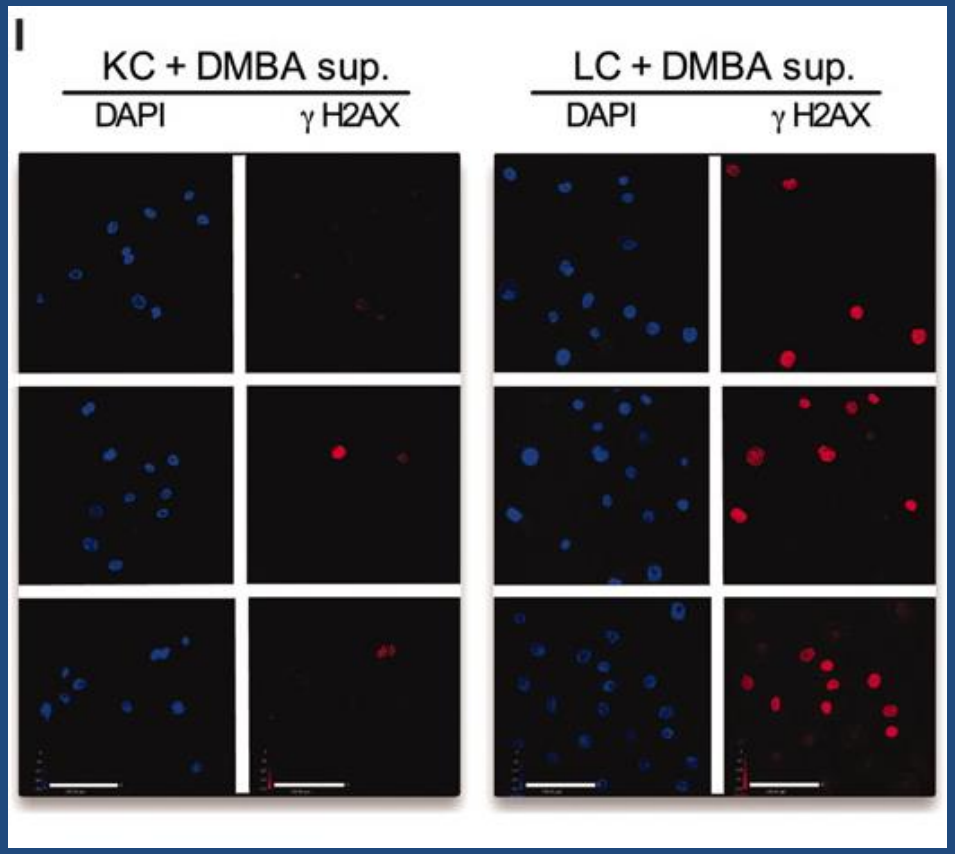


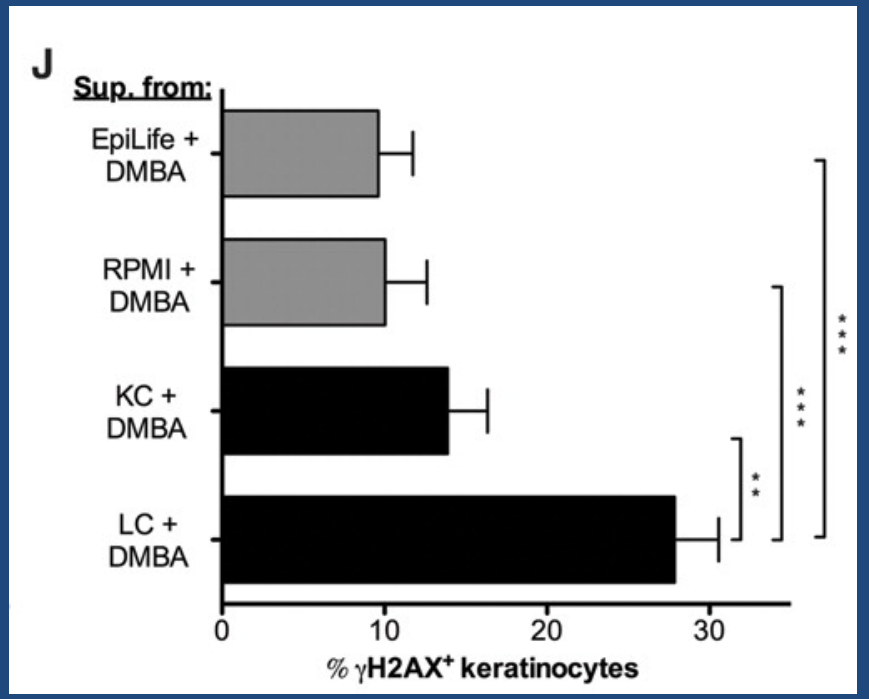
BG Modi et al., Science 335 (2012) 104-108

Increased DNA damage (Hras mutation/ H2AX+) in keratinocytes by supernatants from LC after DMBA incubation compared to keratinocytes

LC + DMBA → Supernatant → Keratinocytes (24h)

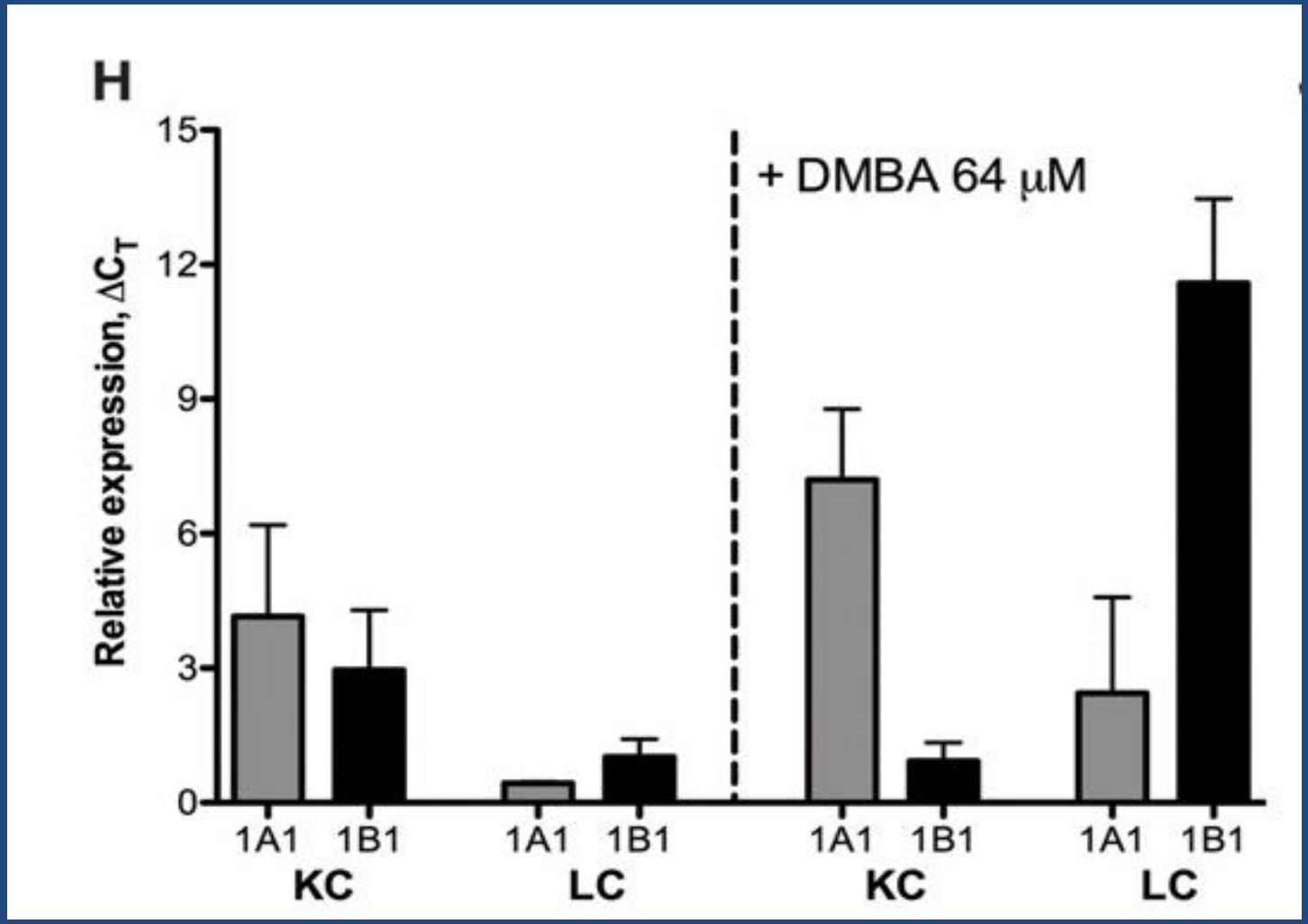
Ke + DMBA → Supernatant → Keratinocytes (24h)





BG Modi et al., *Science* 335 (2012) 104-108

AhR responsive CYPs in muLC



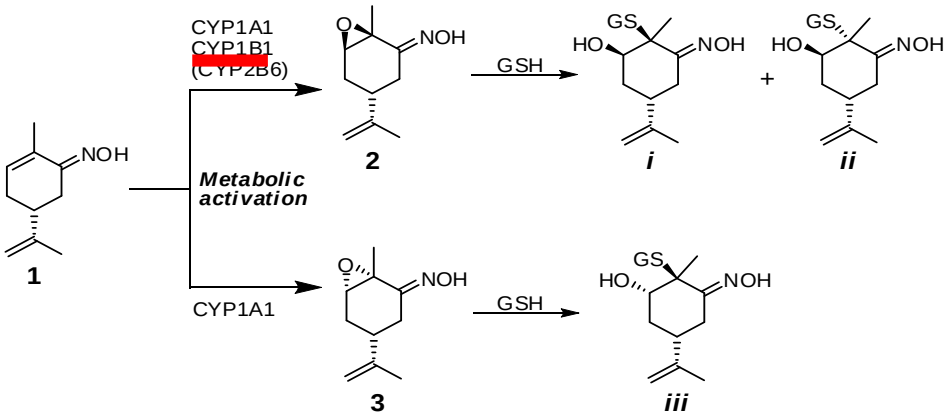
BG Modi et al., Science 335 (2012) 104-108

Metabolic activation of Prophapten R-Carvoxime

Cutaneous Metabolic Activation of Carvoxime, a Self-Activating, Skin-Sensitizing Prohapten

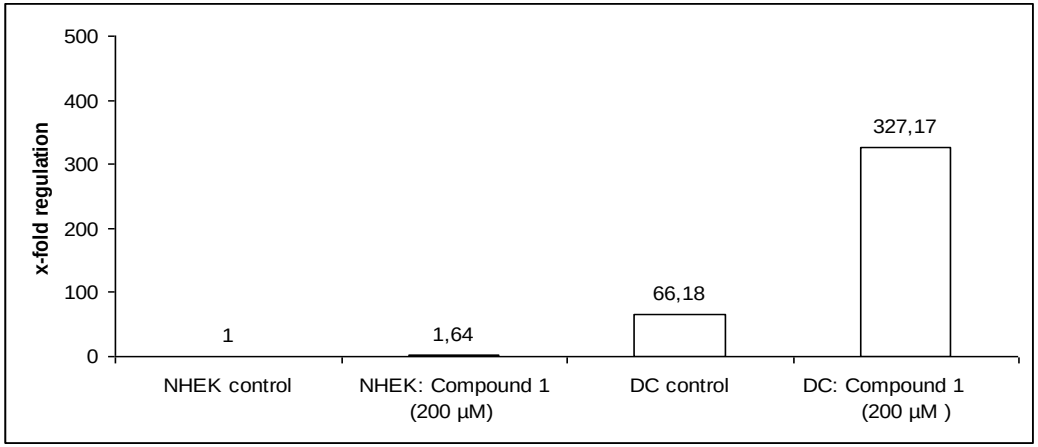
Hagen Ott[a], Moa Andresen Bergström[b], Ruth Heise[a], Claudia Skazik[a], Gabriele Zwadlo-Klarwasser[c], Hans F. Merk[a], Jens M. Baron[a] and Ann-Therese Karlberg[b]

Chem Res Toxicol, 22(2): 399-405, 2009



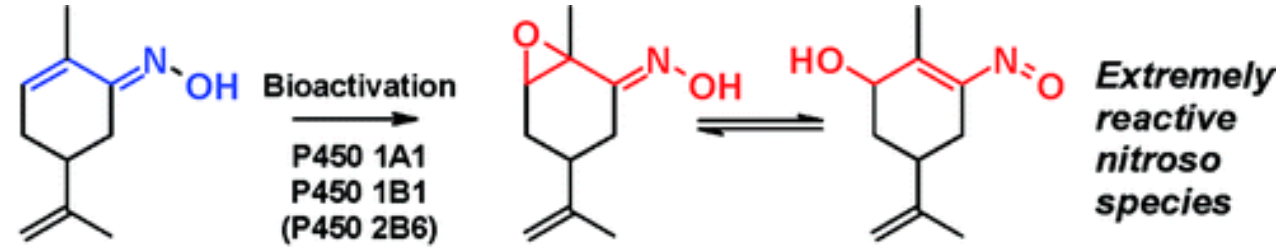
CYP mediated epoxidation of the **prohapten** α,β-unsaturated oxime **R-carvoxime** and subsequent conjugation with glutathione (GSH)

Prohapten (R-Carvoxime) can **stimulate** its CYP-mediated **metabolism (CYP 1B1)**



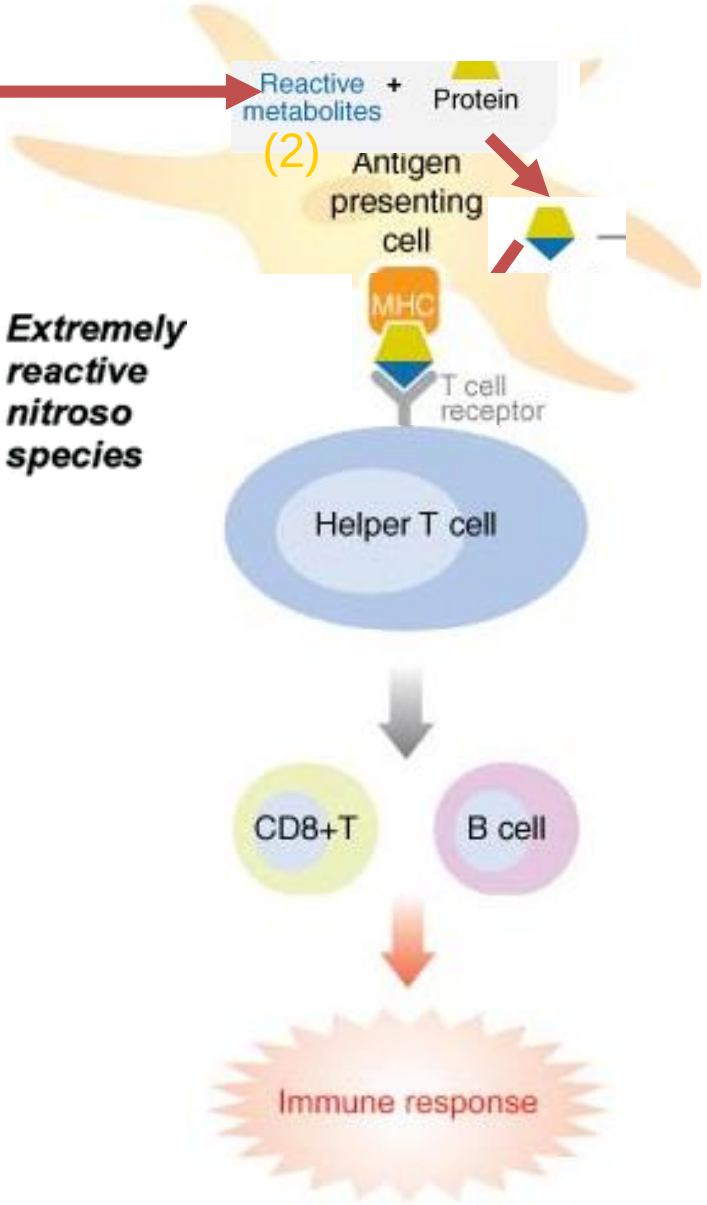
EXZELLENZ RWTH AACHEN

Carvoxime (1)



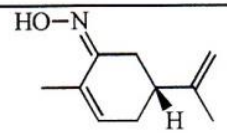
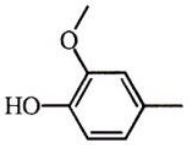
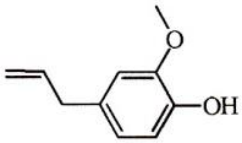
Extremely reactive nitroso species

Prohapten Induces P450 1B1 in antigen-presenting cells



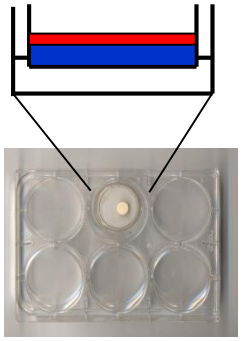
Adapted from Uetrecht et al., 2007 and H Ott et al, 2009

NRF2 activation +/- S9 in Keratinosens

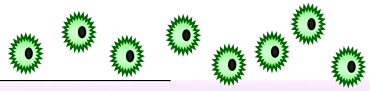
		LLNA EC3 (%)	I_{max} (fold induction)		EC2 (µM)		IC50 (µM)	
			No S9	With S9	No S9	With S9	No S9	With S9
	Carvone oxime[1]	0.6	0.99	1.1	n.i.	n.i.	551	520
	Creosol	5.8	1.1	3.6	n.i. [1)]	230.1	941.0	>2000
	Eugenol	12.9	1.7	3.4	n.i.	369.1	1239.5	1505.2

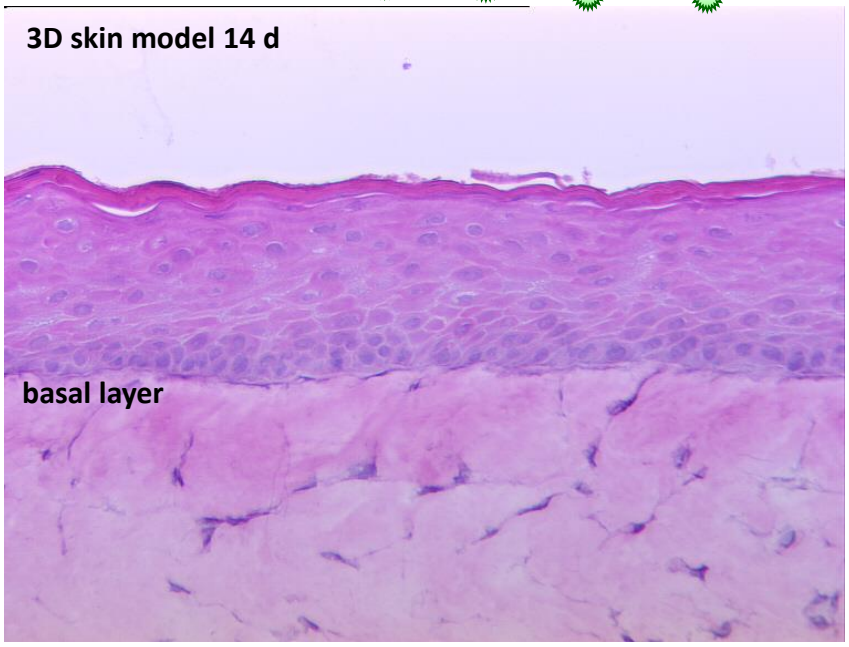
A Natsch & T Haupt; Tox Sci 135(2), 356–368 2013

3D skin models



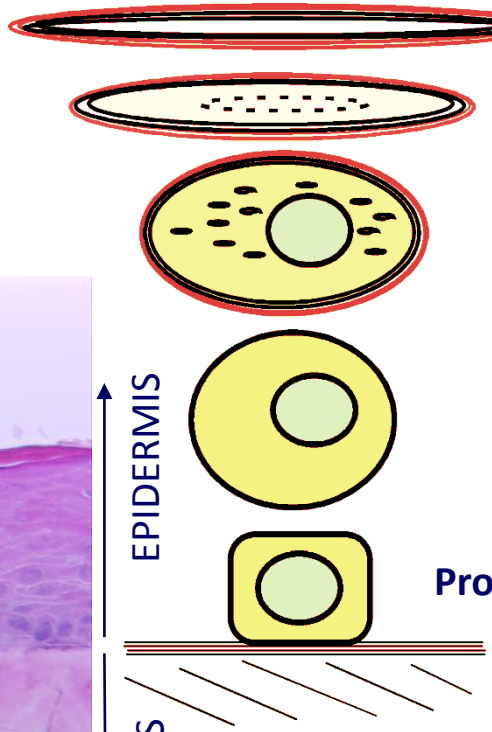
DENDRITIC CELLS





EPIDERMIS

DERMIS



Differentiation

CORNEOCYTES
(anucleated squamous)

TRANSITIONAL CELLS

GRANULAR CELLS
(terminally differentiating)

SPINOUS CELLS
(newly differentiating)

BASAL CELLS
(proliferating & stem cells)

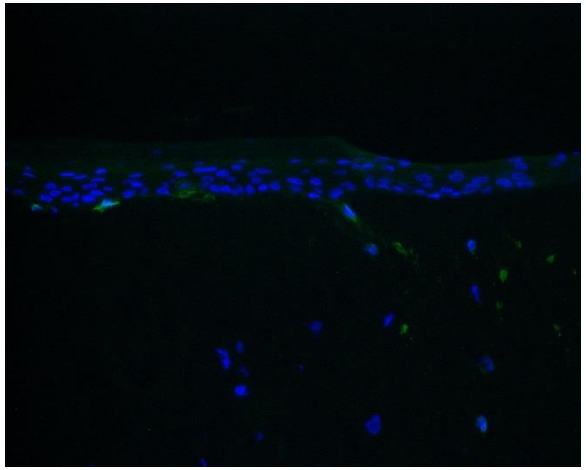
Proliferation

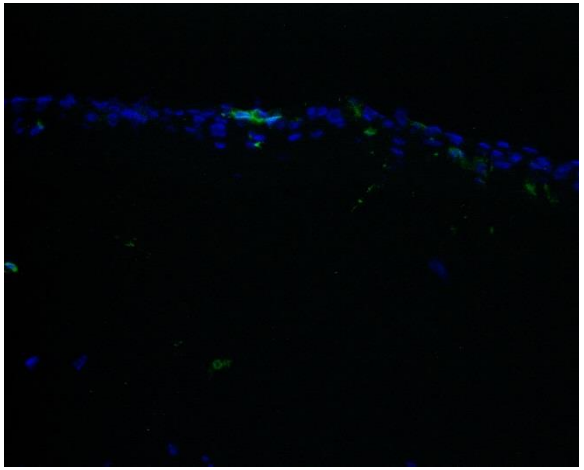
3D4/11 6d 3d skin model with DC

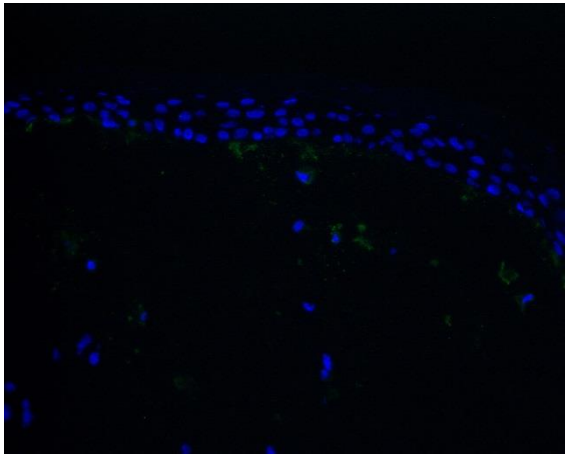
CD1a

CD80

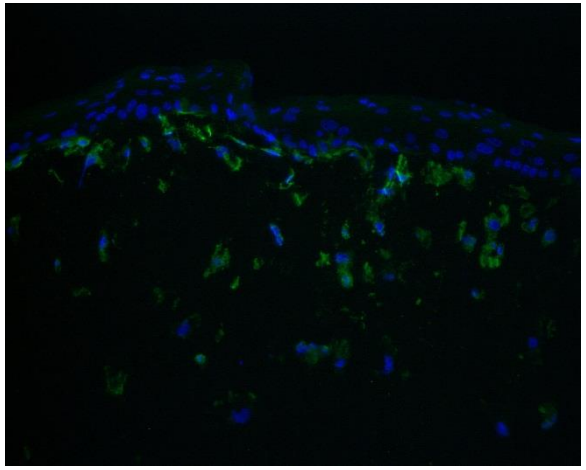
HLADR

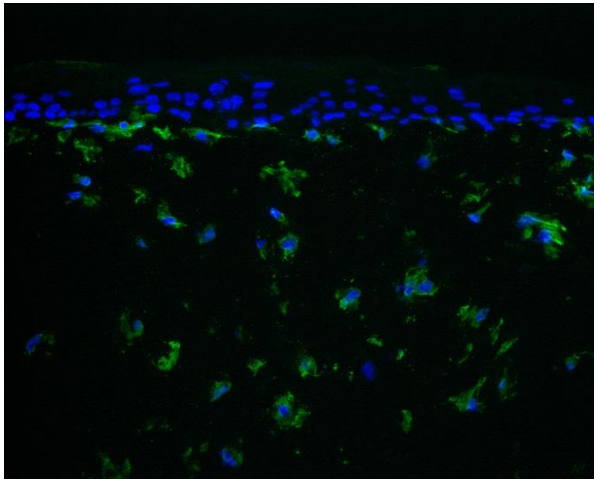


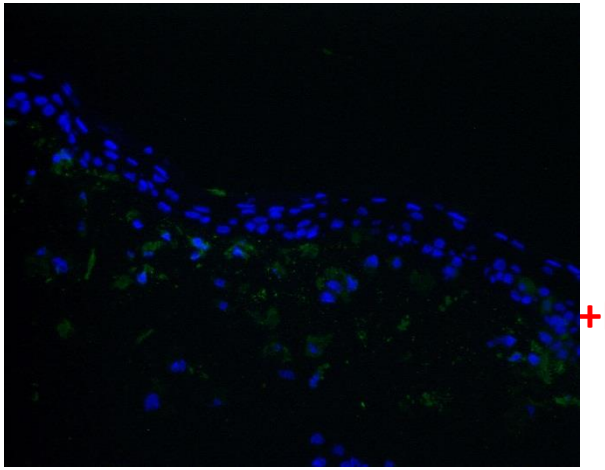




-MIF

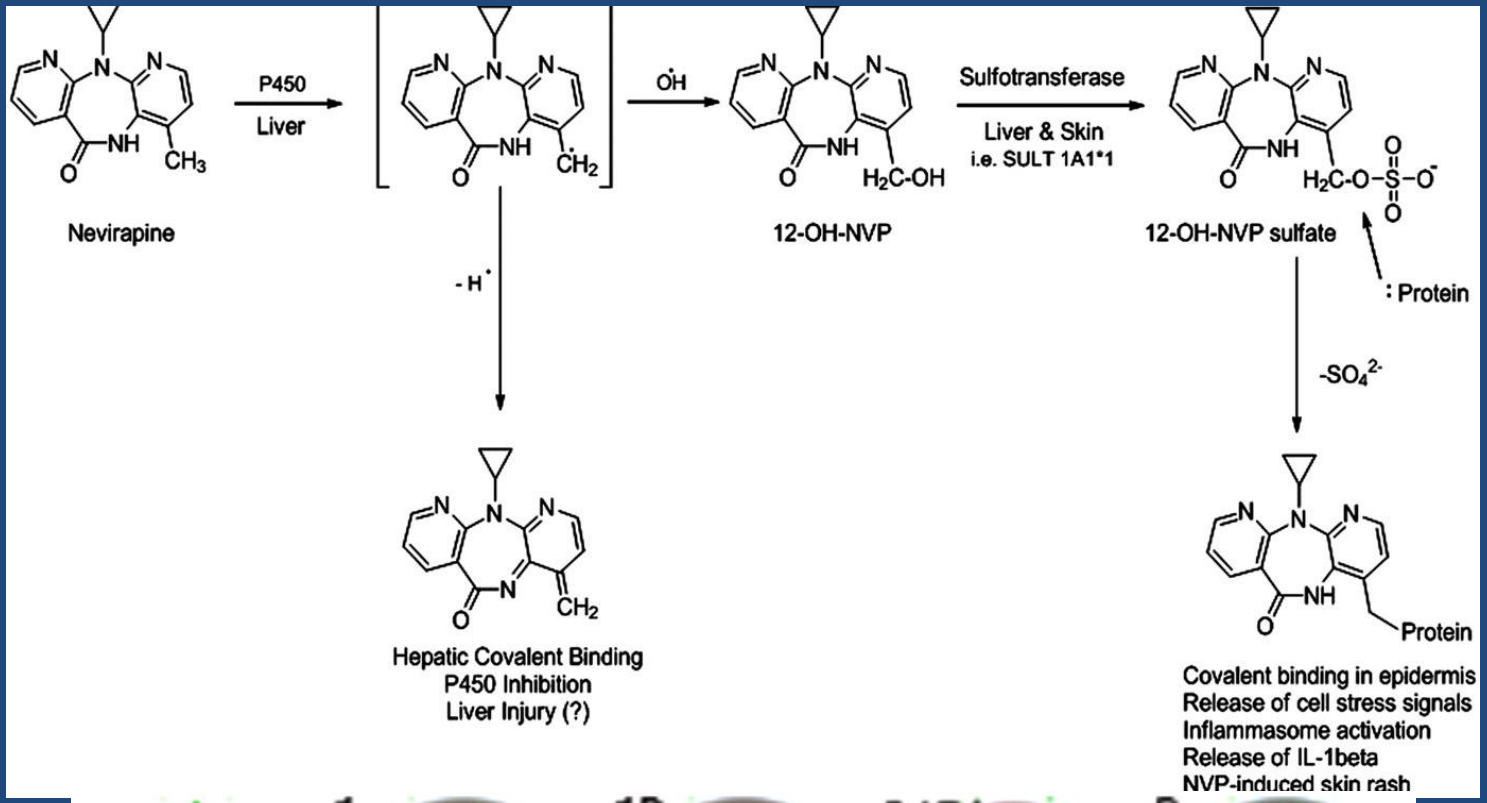


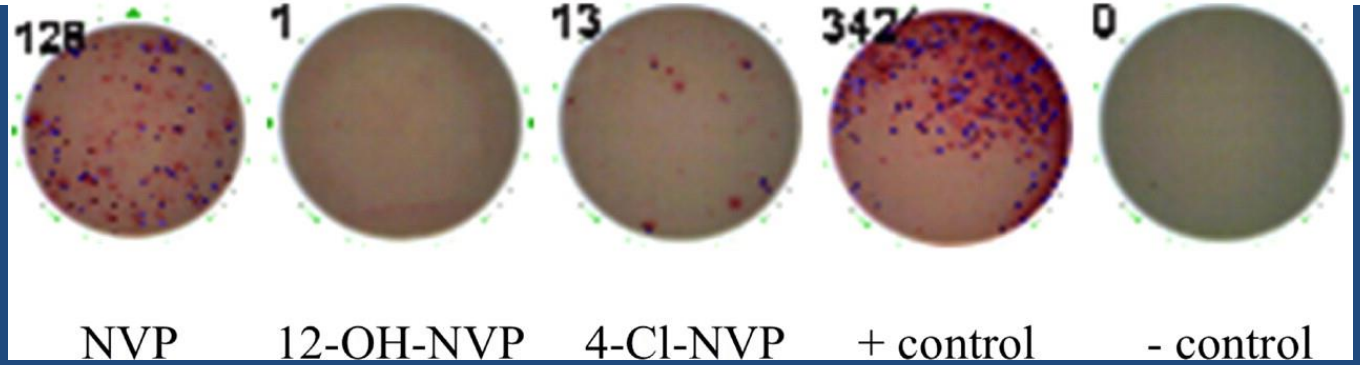




+MIF

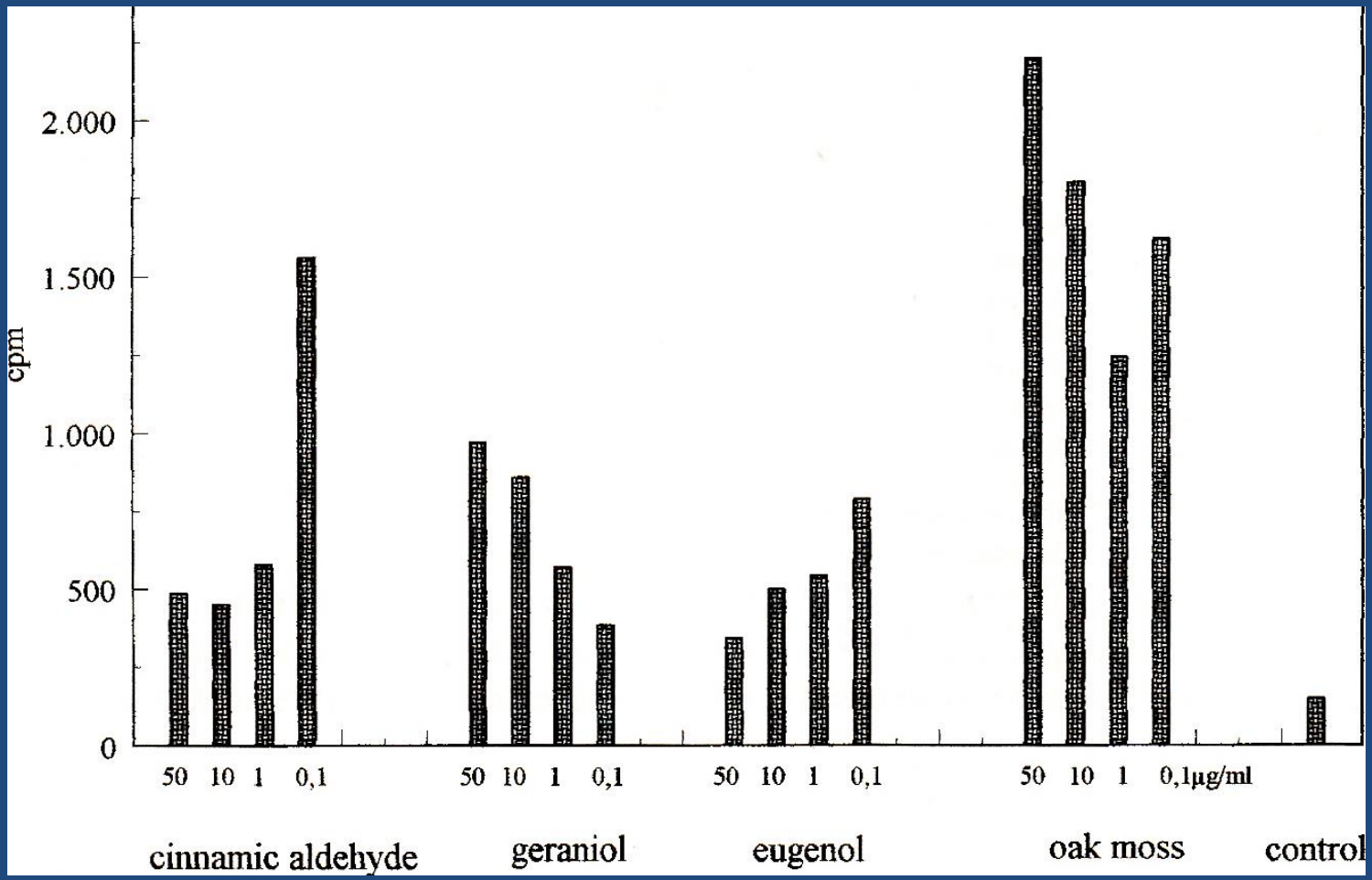
Nevirapine-Metabolism in Liver and skin





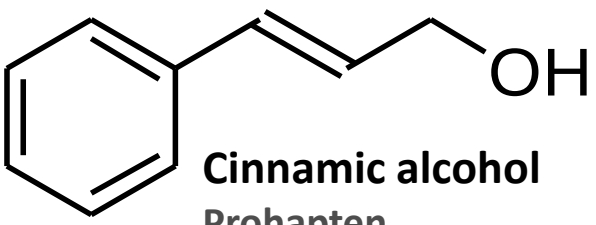
Sharma & Uetrecht, 2014

Dose-dependent proliferation of lymphocytes to fragrances



S Sieben, B Blömeke, HF Merk, In: P. Elsner, HF Merk, HI Maibach Cosmetics Springer, Heidelberg, 1999, pp. 226 - 240

Activation of cinnamic alcohol is mediated by CYPs expressed in skin cells



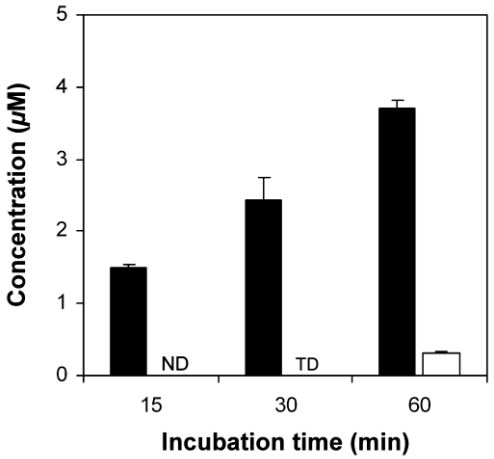
Cinnamic alcohol
Prohapten

Incubation with skin specific rhCYP cocktail

+

ADH*

Detection of cinnamic aldehyde(■) and cinnamic acid (□) by LC-MS



**CK Smith et al., TaP 168 (2000) 189-199*
J Invest Dermatol, 127: 1145–1153, 2007
Skin Pharmacol Physiol, 23(4):213-224, 2010

Human moDC in vitro assay

Characterization of the Sensitizing Potential of Chemicals by *In Vitro* Analysis of Dendritic Cell Activation and Skin Penetration

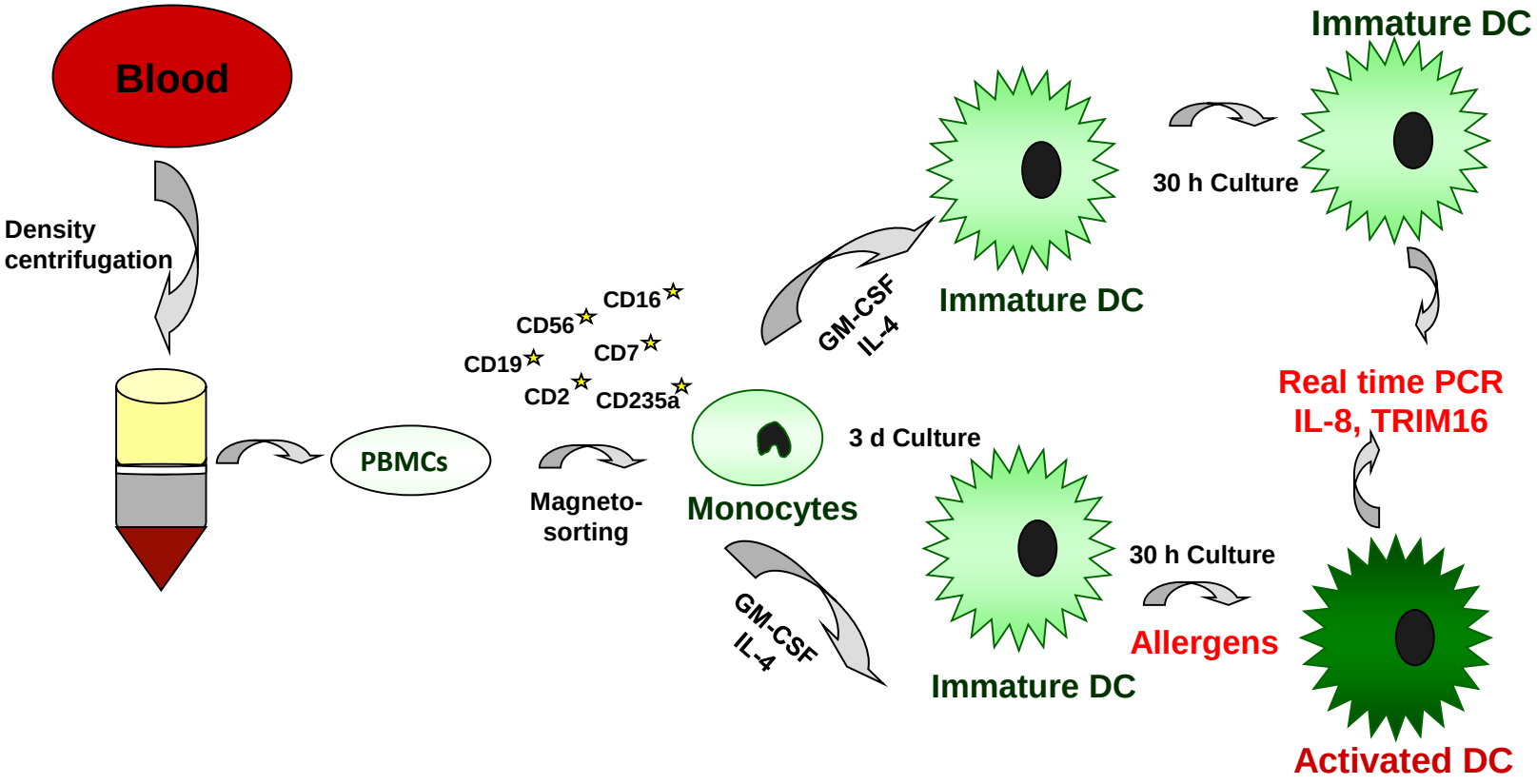
Pierre Aeby,* Christoph Wyss,* Heinz Beck,* Peter Griem,§ Heike Scheffler,† and Carsten Goebel†

J Invest Dermatol 122:1154–1164, 2004

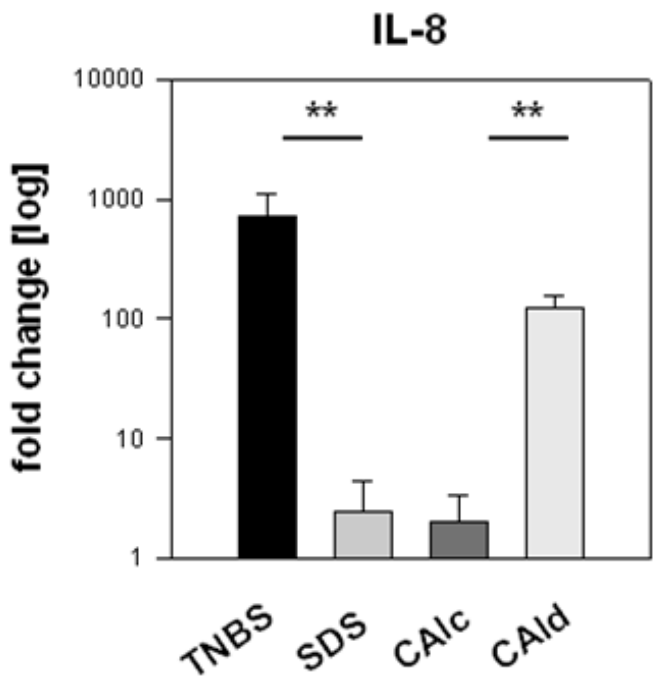
High-Resolution Transcriptional Profiling of Chemical-Stimulated Dendritic Cells Identifies Immunogenic Contact Allergens, but Not Prohaptens

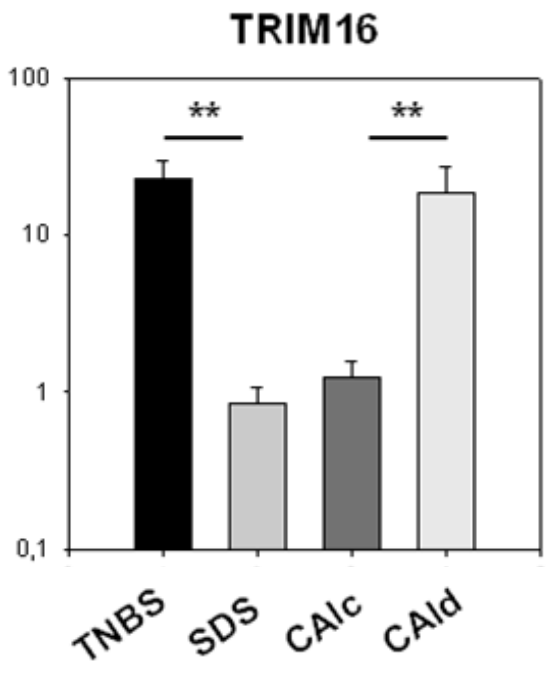
H. Ott[a] T. Wiederholt[a] M. Andresen Bergström[b] R. Heise[a] C. Skazik[a] K. Czaja[a] Y. Marquardt[a] A.-T. Karlberg[b] H.-F. Merk[a] J.M. Baron[a]

Skin Pharmacol Physiol 2010;23:213–224



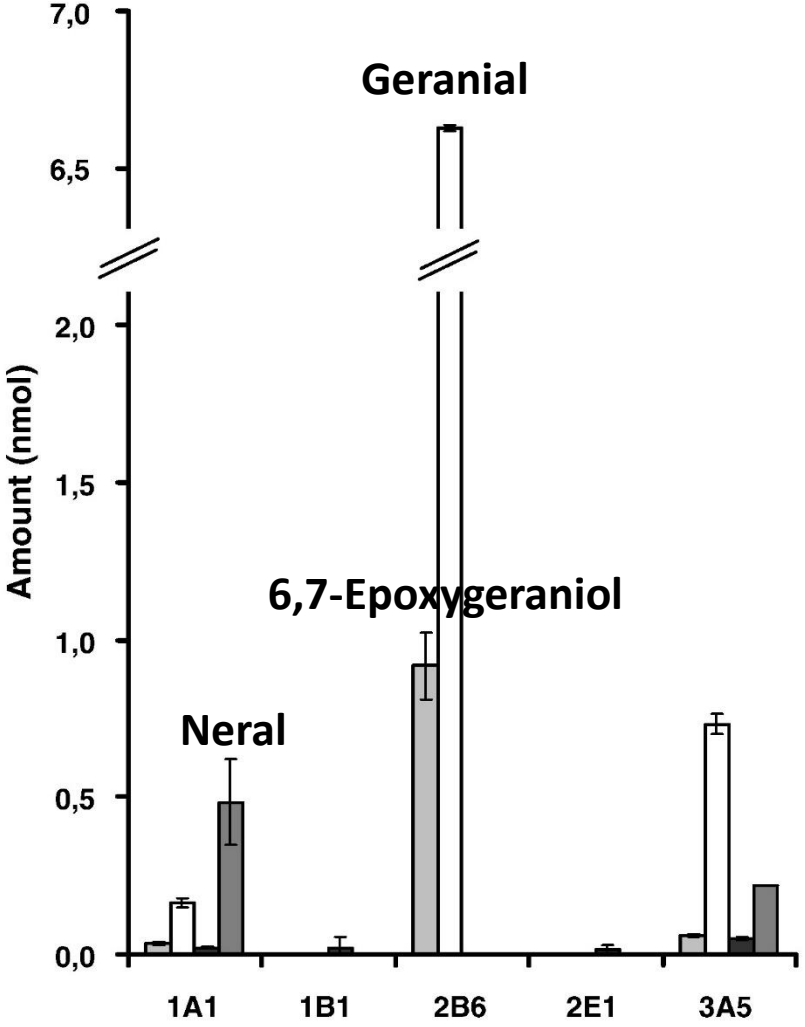
Influence of prohaptens and haptens on the activation of immature dendritic cells

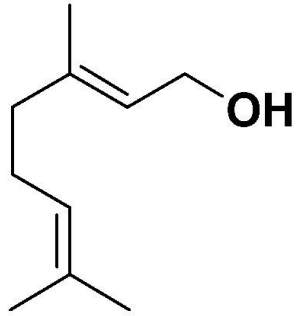




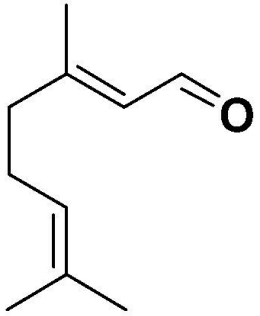
Skin Pharmacol Physiol, 23(4):213-224, 2010

Autooxidation and CYP-metabolism important in sensitization to geraniol

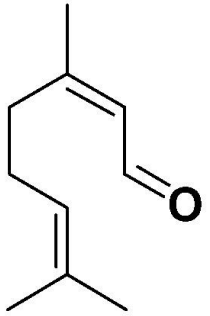




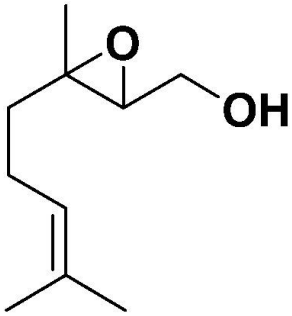
Geraniol



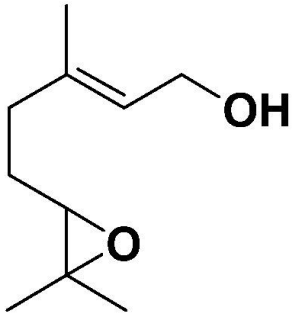
Geranial



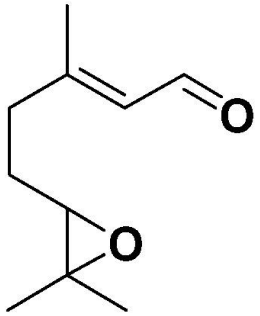
Neral



2,3-Epoxy-geraniol



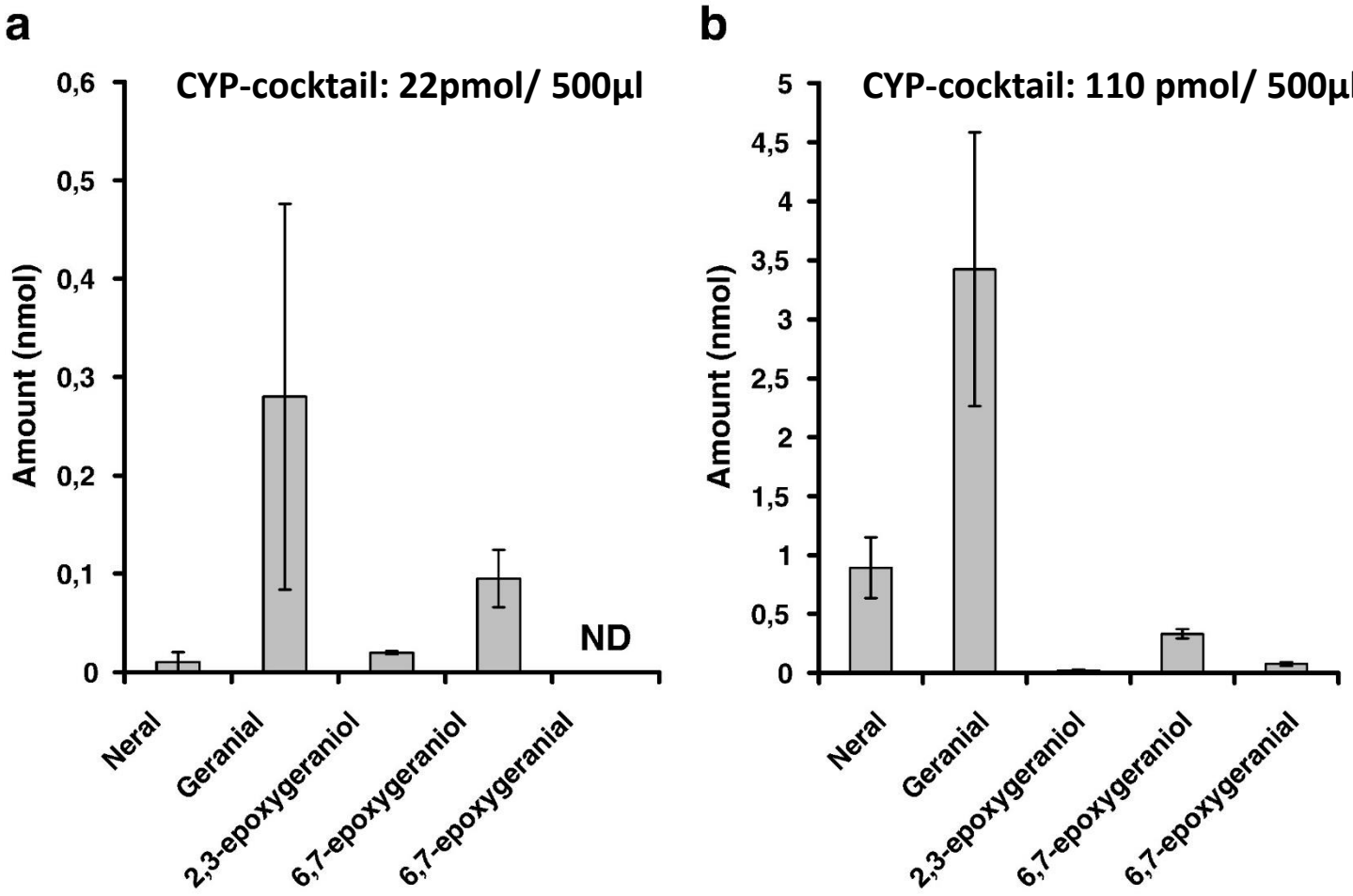
6,7-Epoxy-geraniol



6,7-Epoxy-geranial

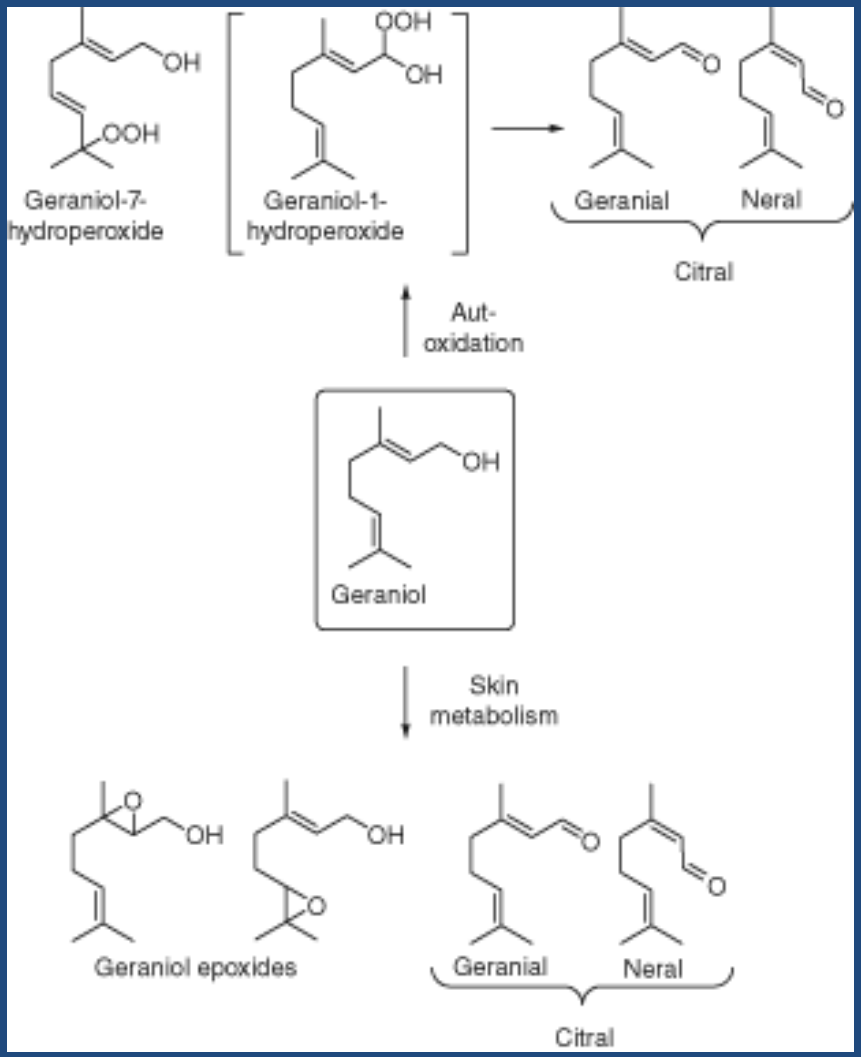
Hagvall L et al., TaP 233 (2008) 308 – 313; CD 67 (2012) 20-27

Autooxidation and CYP-metabolism important in sensitization to geraniol



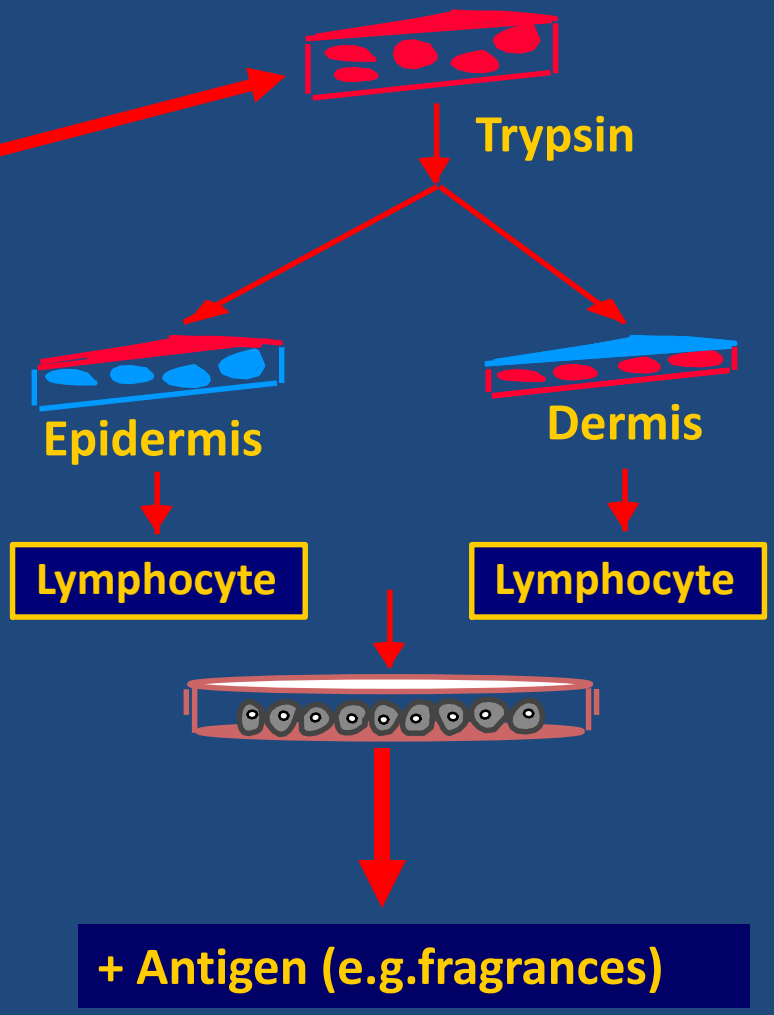
Hagvall L et al., TaP 233 (2008) 308 – 313; CD 67 (2012) 20-27

Geraniol: Pre- and Prohapten

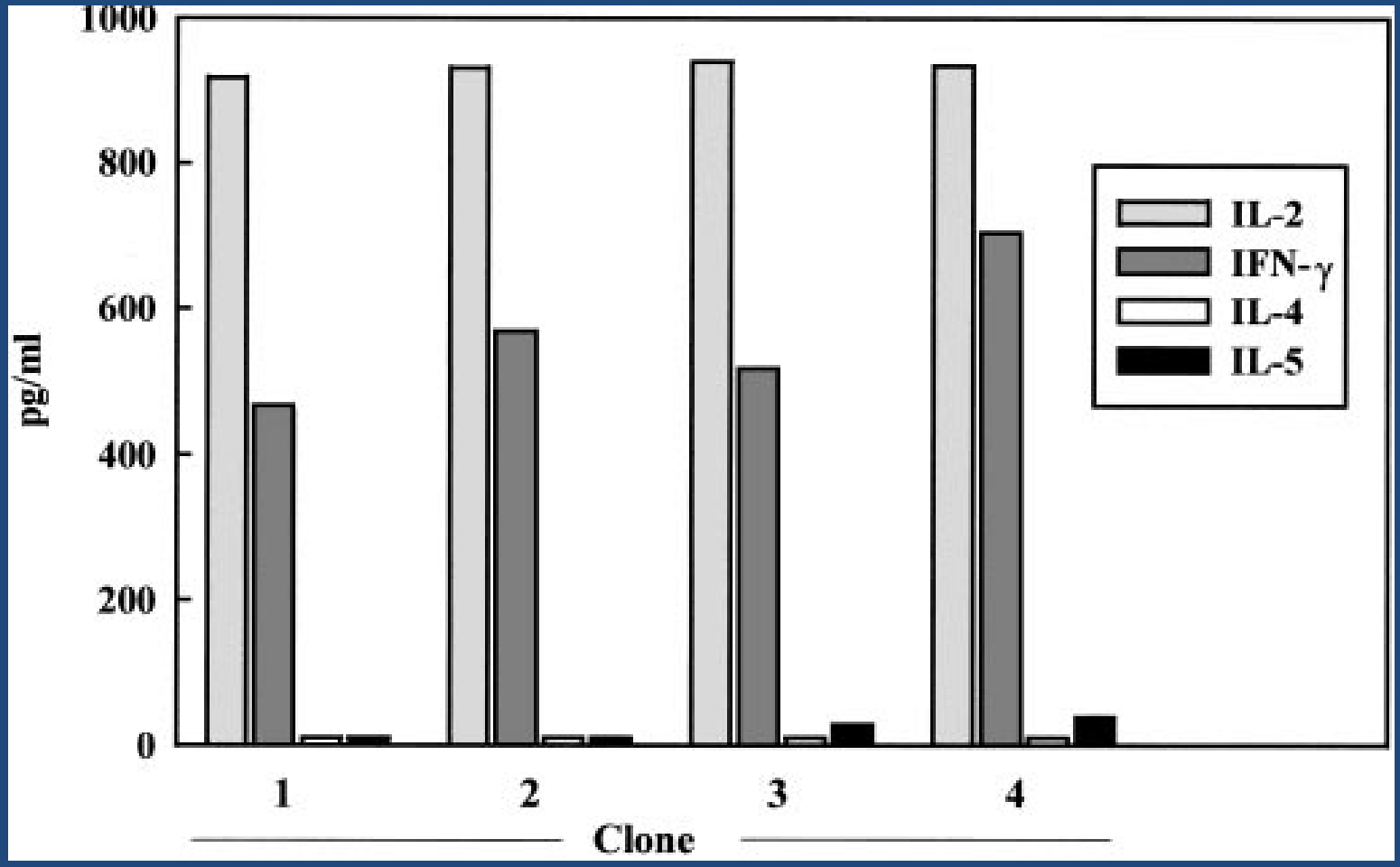


Hagvall L et al., CD 2014

Isolation and cloning of lesional T-lymphocytes



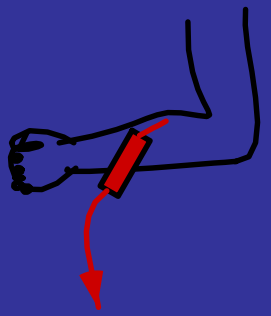
Cytokine release of eugenol-specific TCC



Sieben et al., TaP 2001

LTT with Fragrance modified CYPs

human liver microsomes
recombinant CYPs
+ fragrances



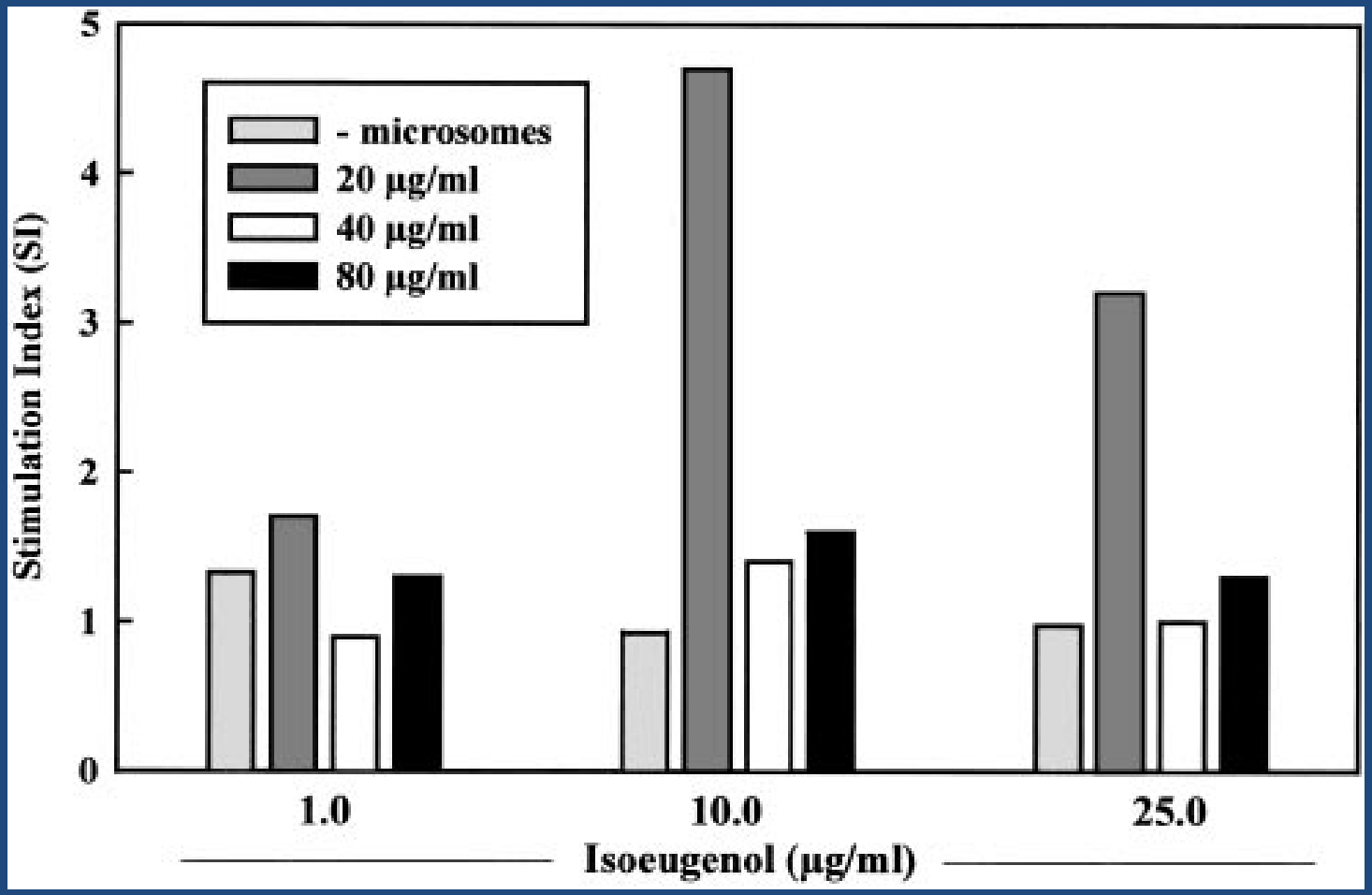
Supernatant

venous blood
PBMC

^{3} H-thymidine

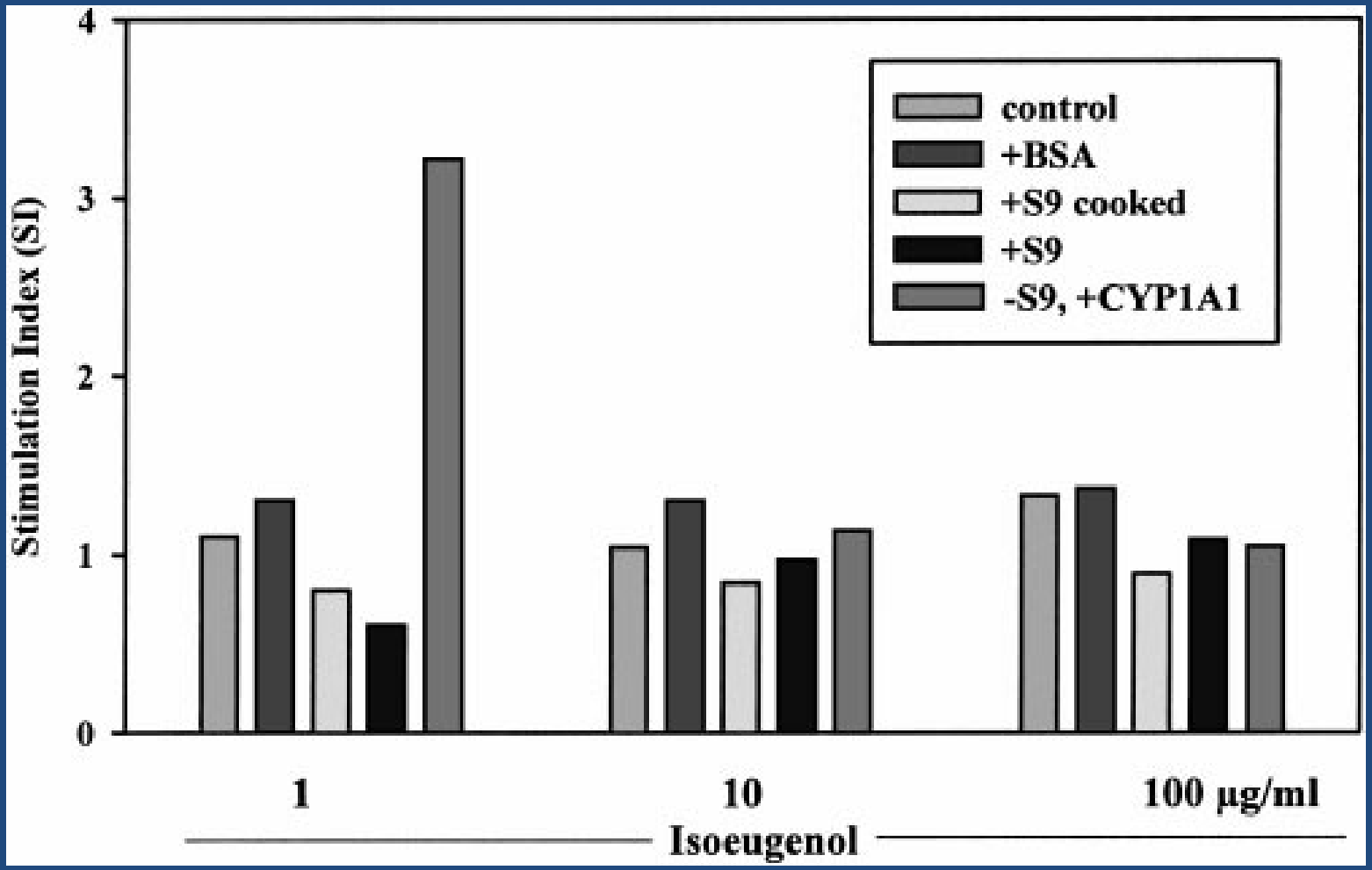
proliferation

Effects of fragrance-modified human CYPs



Sieben et al., TaP 2001

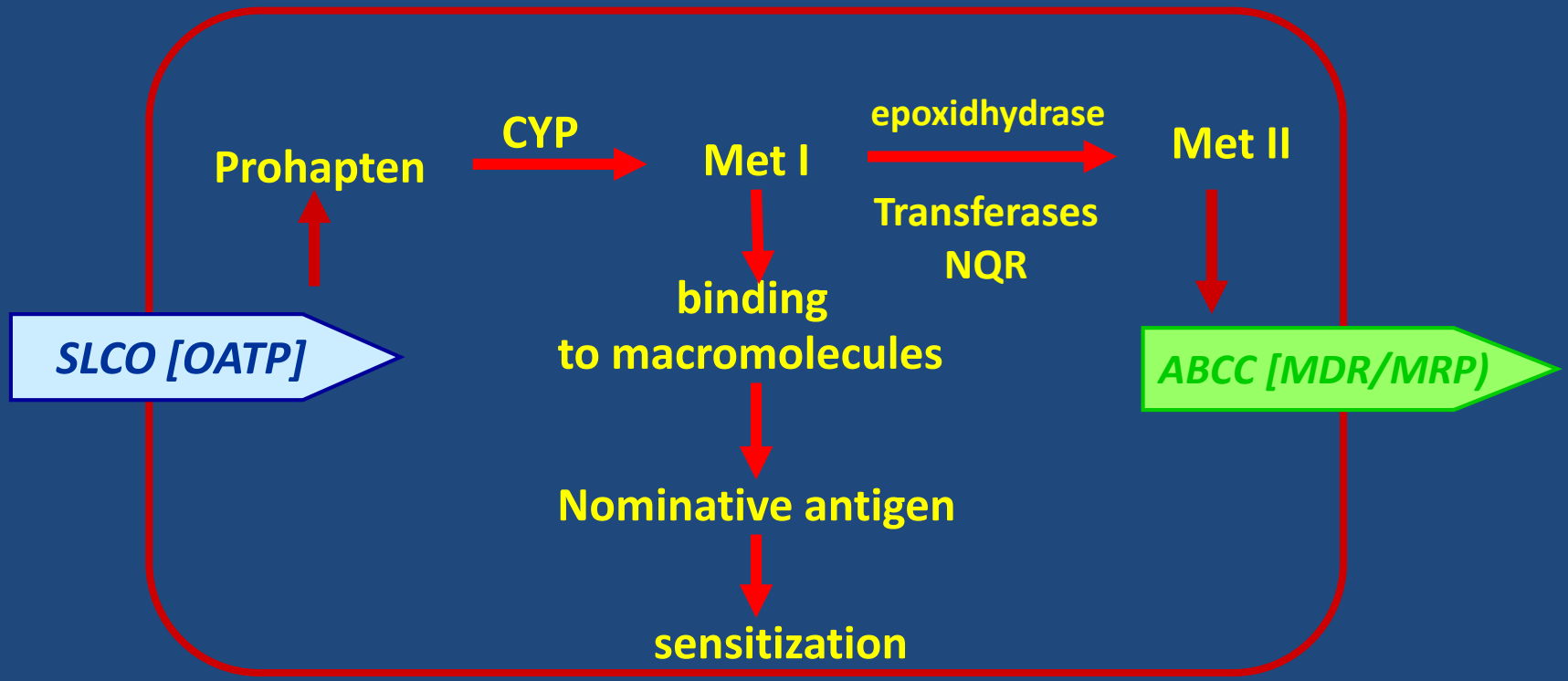
Effects of isoeugenol-modified recombinant CYP1A1 or human liver microsomes compared to effects of cooked or irrelevant protein



Sieben et al., TaP 2001

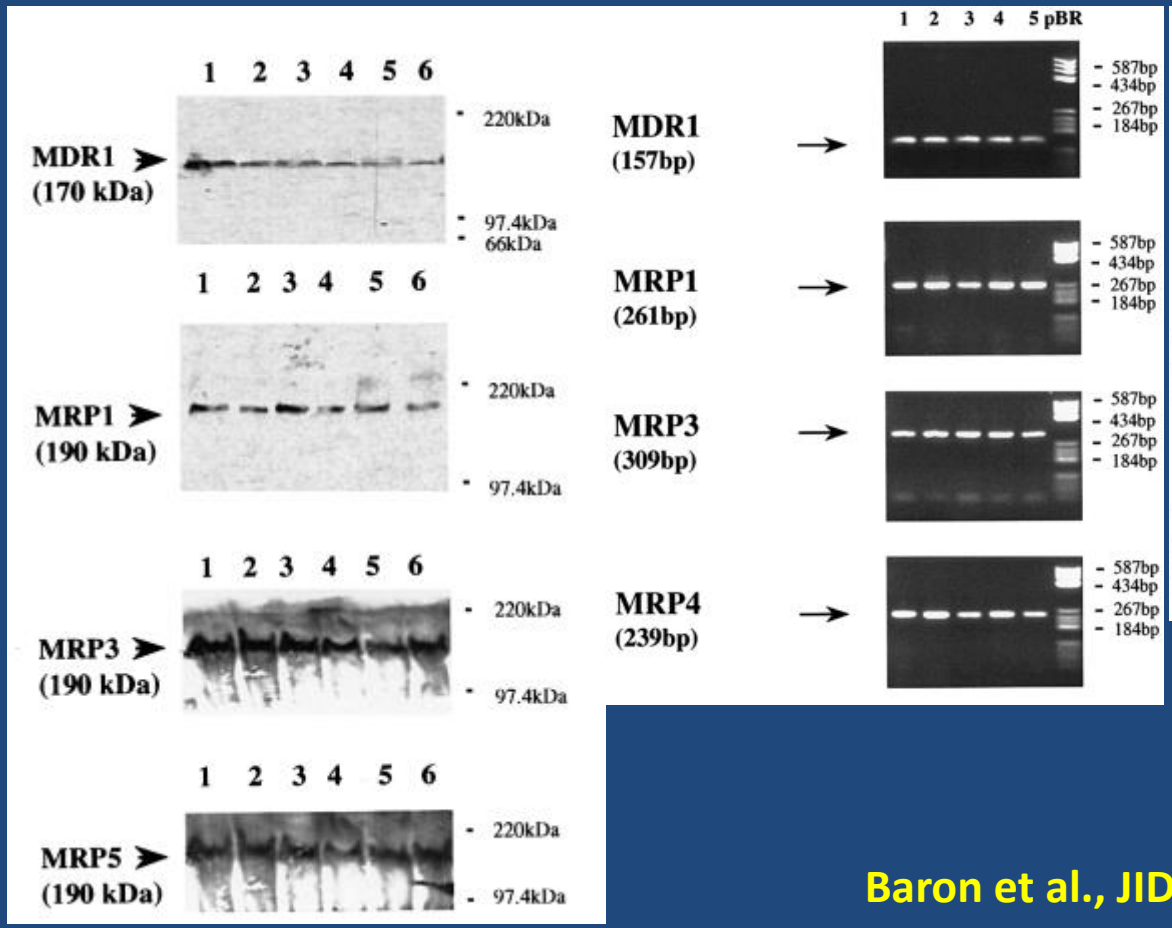
Metabolism/ Toxicity of xenobiotica

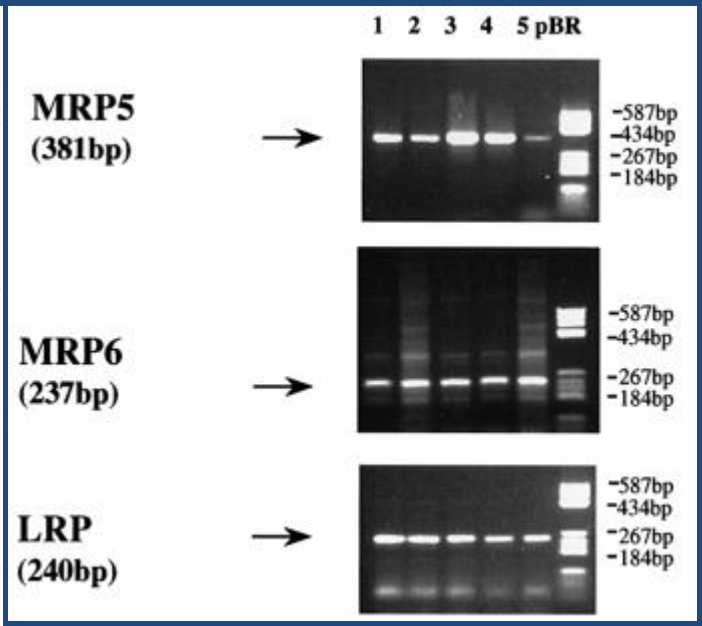
Phase I: CYP – Phase II: EH/ Transferases –
Phase III: Transporter proteins



ABCC: ATP binding cassette C transporters (MRP/ MDR)
SLCO: solute carrier organic anion transporter (OATP)

Transporter proteins in human keratinocytes



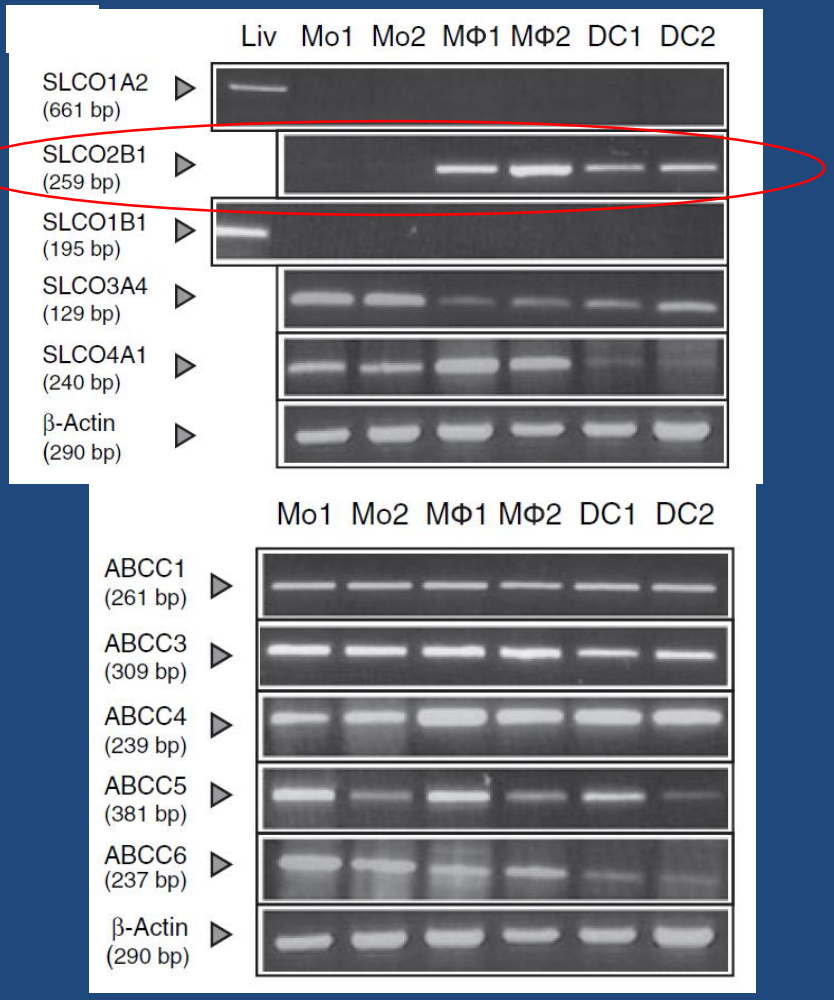


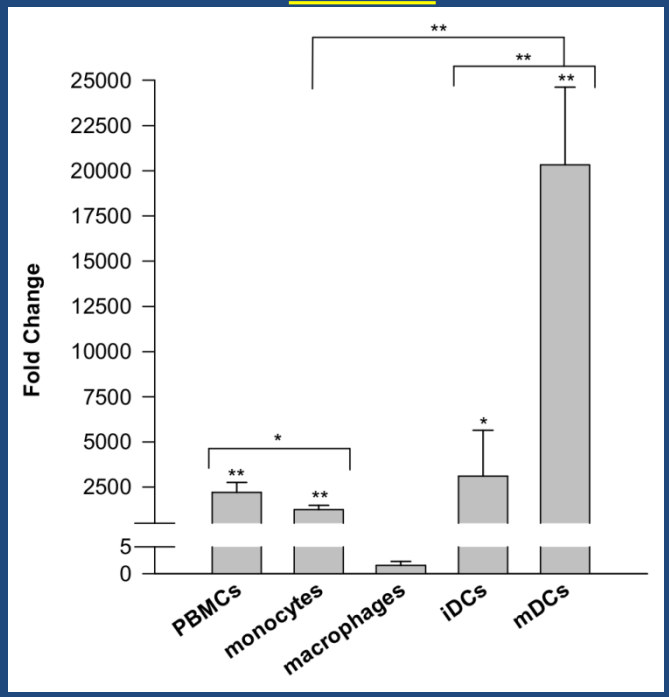
Baron et al., JID 116 (2001) 541-548

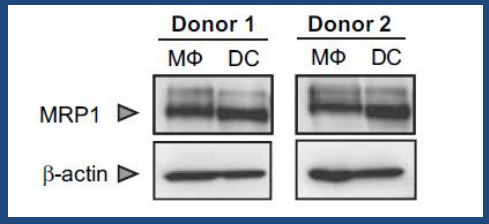
Expression of ABCC- and SLCO- Transporter in monocytes/ macrophages

SLCO/ OATP

ABCC/ MRP



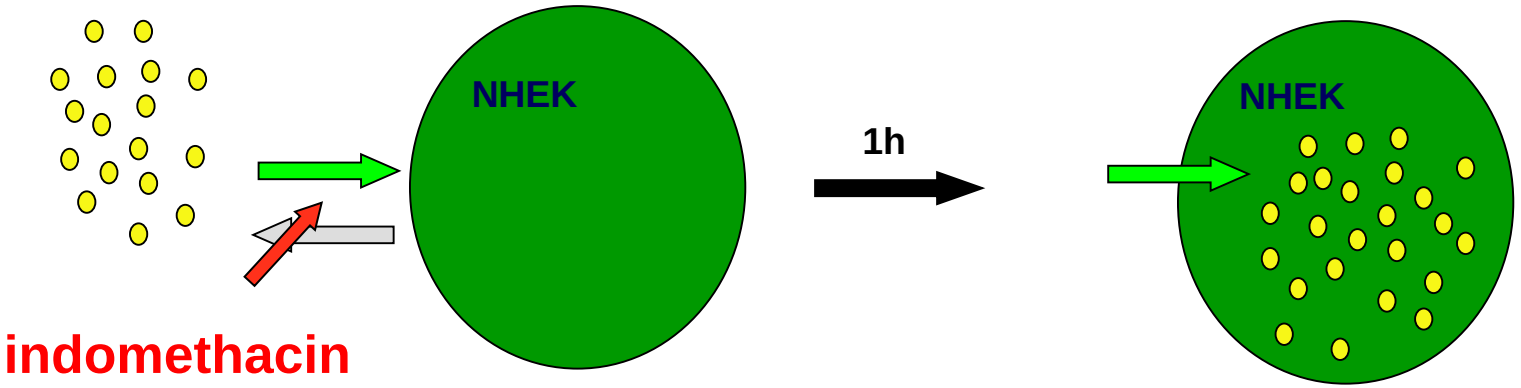




Skazik et al., 2008

Liv: Leber; Mo: Monocytes; MΦ: Macrophages; PBMCs: peripheral blood mononuclear cells; i/mDCs: immature/ mature dendritic Cells

Transport assay



indomethacin

- **specific MRP inhibitor**
- **concentration of 1 mM and 200 µM**

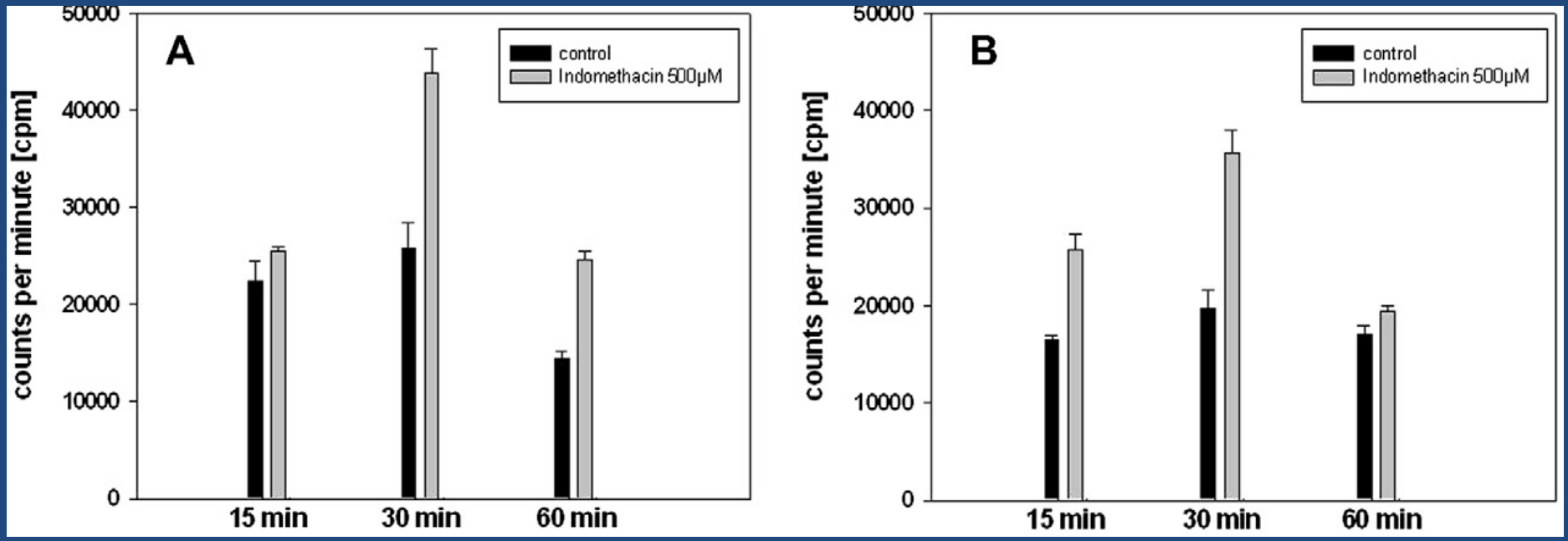
scintillation buffer

Measurement of cell-associated radioactivity

Inhibitory effect of indomethacin on MRP-mediated efflux of contact allergens in moDCs

Eugenol

Isoeugenol

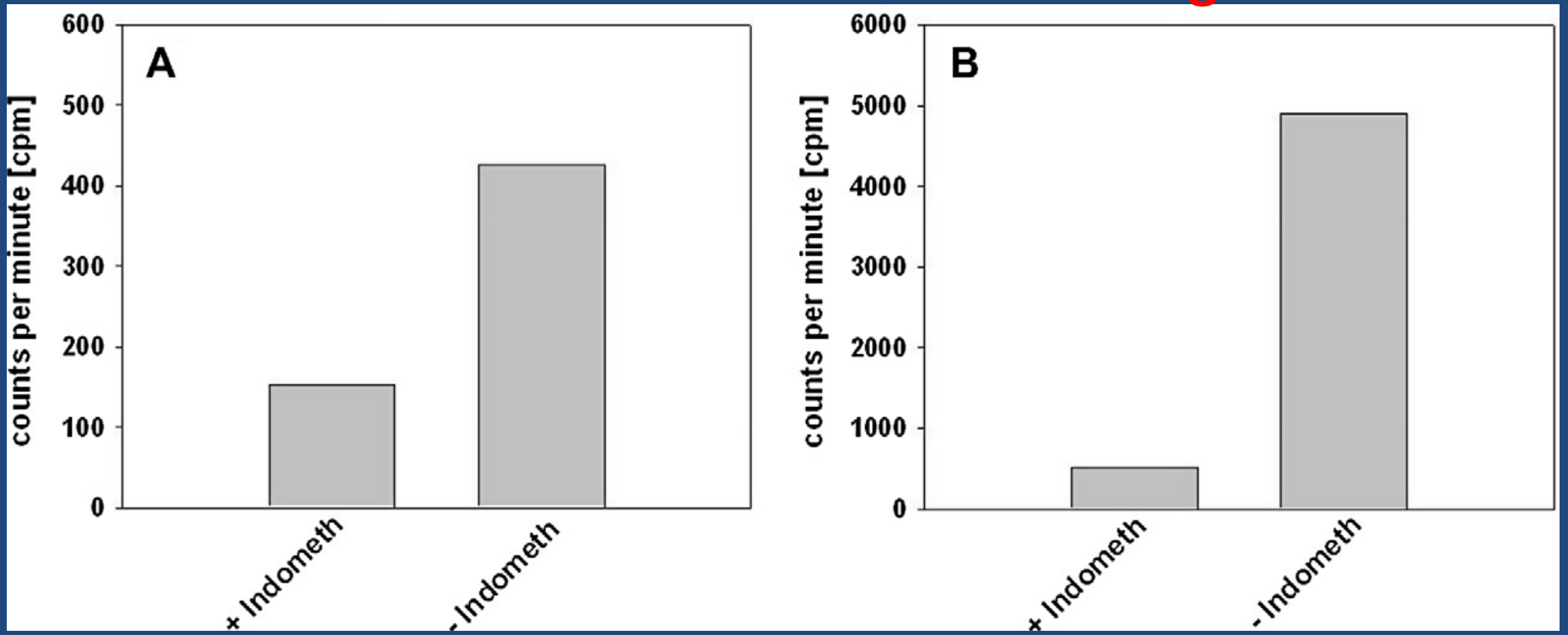


Skazik et al., TaP 2012

Vesicular transport assay for MRP1: Transport of eugenol is mediated by MRP1

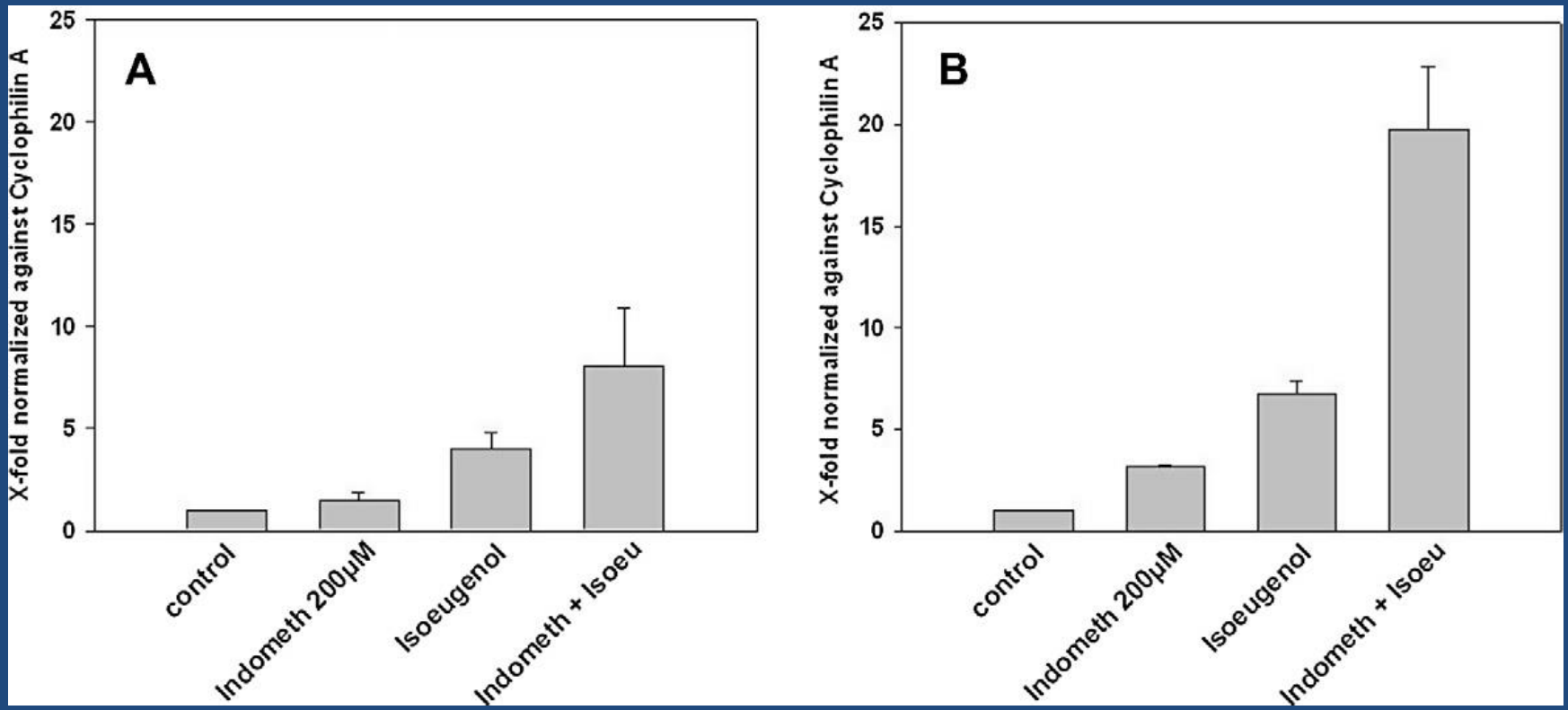
LTC4

Eugenol



Skazik et al., TaP 2012

MoDC-based in vitro assay IL-8 (A) and TRIM 16 (B) mRNA expression



Skazik et al., TaP 2012

Summary: The skin as a metabolizing organ of pro-haptens

1. Multiple CYPs are present in keratinocyte
2. CYP 1A1 is mainly localised in basal keratinocytes and hair follicle keratinocytes
3. The main CYP in antigen-presenting cells (APCs) is CYP 1B1
4. Carvoxime is CYP 1B1 dependently metabolized in APCs
5. Proliferation of lesional T-lymphocyte clones of +patch test reactions to eugenol and isoeugenol is enhanced by CYP-modified fragrances
6. Geraniol can act as pre- and prohapten.
7. Multiple transporter molecules are present in keratinocytes and APCs/ moDCs.
8. The inhibition of MDR leads to a higher concentration of eugenol in keratinocytes and mo-DCs and augments IL8- and TRIM 16 B mRNA formation in moDCs