

Haut: Zielorgan allergischer Reaktionen auf kleinmolekulare Substanzen

Hans F Merk

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1968: Beginn der KoKo

- **Präsident USA: Lyndon B Johnson**
- **Bundespräsident: Heinrich Lübke – Gustav Heinemann**
- **Bundeskanzler: Kurt Georg Kiesinger**
- **Nobelpreis 1977:**
 - Robert W Holley : Isolierung einer t-RNA**
 - Marshall W Nirenberg: PolyU-Experiment**
 - Har G Khorana: gelang 1970 als erstem die künstliche Synthese eines Gens**

STUDIES ON THE SENSITIZATION OF ANIMALS WITH SIMPLE CHEMICAL COMPOUNDS*

By K. LANDSTEINER, M.D., AND JOHN JACOBS, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research)

PLATE 30

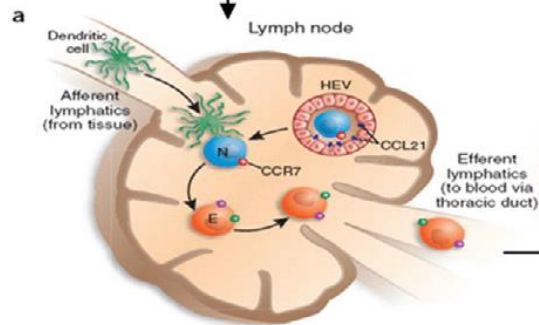
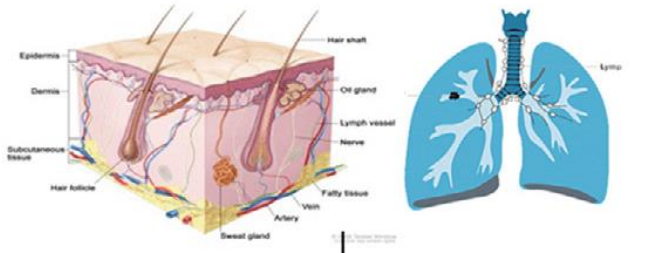
(Received for publication, January 25, 1935)



Asthma (IgE) durch kleinemolekulare Substanzen nur nach Sensibilisierung (mit Ekzemreaktion) über die Haut

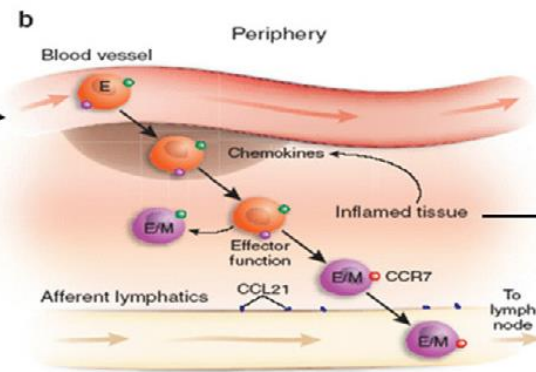
Skin induction exposure (Ia)

Lung priming exposure (Ib)



Lymph node priming & Th-cell polarization
Expression of cytokines due to irritation and/or sensitization

Systemic sensitization (II)

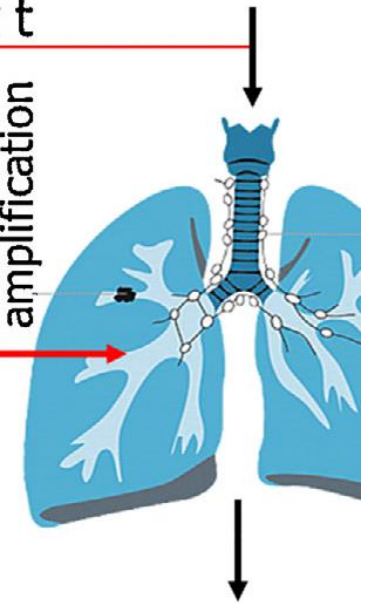


Expression of pre-disposing factors & primed Th-cells

exposures (III)

$\Sigma C \times t$

amplification



Integrating phenotypes of diisocyanate asthma:

- Immediate response
- Delayed response
- Neutrophils in BAL
- Exhaled nitric oxide



Stephen Rothman Medal (courtesy of Georg Stingl, MD, Vienna, Austria; recipient of the award in 2003)

Stephen Rothman and the 60th anniversary of the publication of his epic textbook [The Physiology and Biochemistry of the Skin](#). In this review, we document our belief that Rothman had a seismic impact on [moving investigative dermatology from a medical backwater to a scientific discipline that can hold its own with any other specialty](#).

WHC Burgdorf & DR Bickers, JID 2015 Apr;135(4):954-959

[Steigleder GK](#) Personal experiences with Stephen Rothman, M.D. [J Am Acad Dermatol](#). 1988 Sep;19(3):596-8.

1966: Human repeated insult patch test/ Albert Kligman

THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
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Vol. 47, No. 5
Printed in U.S.A.

THE IDENTIFICATION OF CONTACT ALLERGENS BY HUMAN ASSAY

III. THE MAXIMIZATION TEST: A PROCEDURE FOR SCREENING AND RATING CONTACT SENSITIZERS*

ALBERT M. KLIGMAN, M.D., PH.D.



**J invest Dermatol
47 (1966) 393 - 409**

1966: Human repeated insult patch test/ Albert Kligman

1. Sitzungsprotokoll KoKo 1968:
...jede absichtliche Sensibilisierung eine Körperverletzung...

www.bfr.bund.de



Bundesinstitut für Risikobewertung

14. Sitzung der BfR-Kommission für kosmetische Mittel

Protokoll der Sitzung vom 6. November 2014

5 Die Auslobung „hypoallergen“

Addendum: der Claim „Hypoallergen“ für kosmetische Produkte

(Prof. Dr. Ulrike Heinrich, Dr. Annemarie Burkhard)

Nach Artikel 3 der Europäischen Kosmetikverordnung (VO(EG) Nr. 1223/2009) müssen die auf dem Markt bereitgestellten kosmetischen Mittel bei normaler oder vernünftigerweise vor-

14. Sitzung der BfR-Kommission für kosmetische Mittel

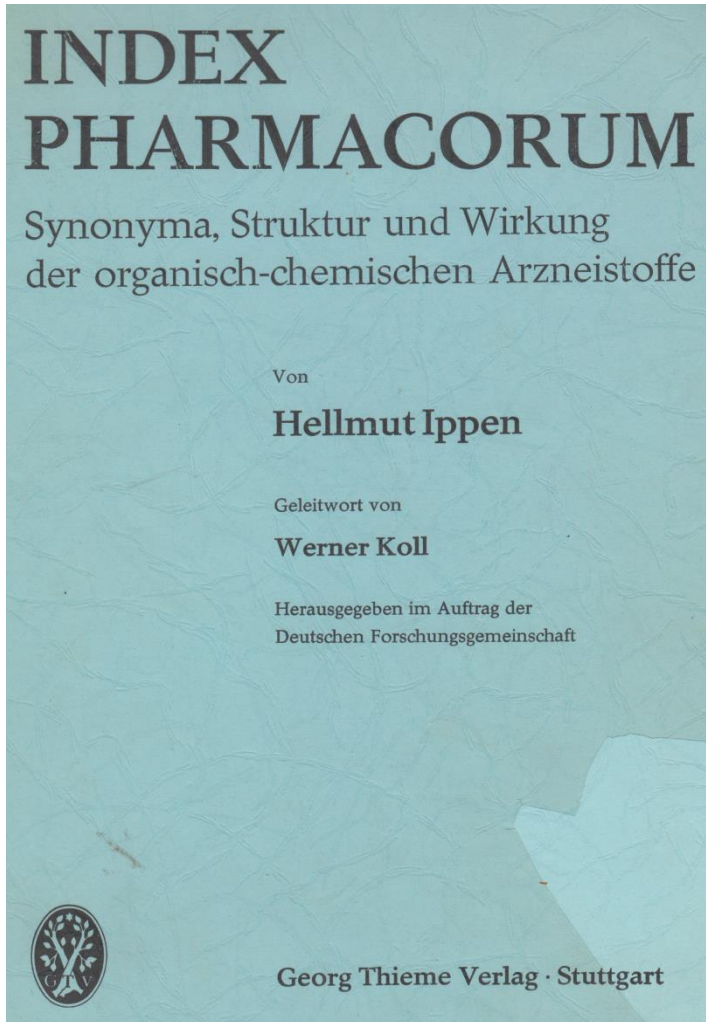
Protokoll der Sitzung vom 6. November 2014

Das SCCNFP hat in einer am 17. Februar 2000 verabschiedeten Opinion (SCCNFP/0068/99 „The predictive testing of potentially cutaneous sensitising cosmetic ingredients or mixtures of ingredients“)^[5] ethische Bedenken gegen den HRIPT formuliert. Dieser Test ist zum Zweck der Belegbarkeit einer Werbeaussage aus ethischen Gründen abzulehnen.

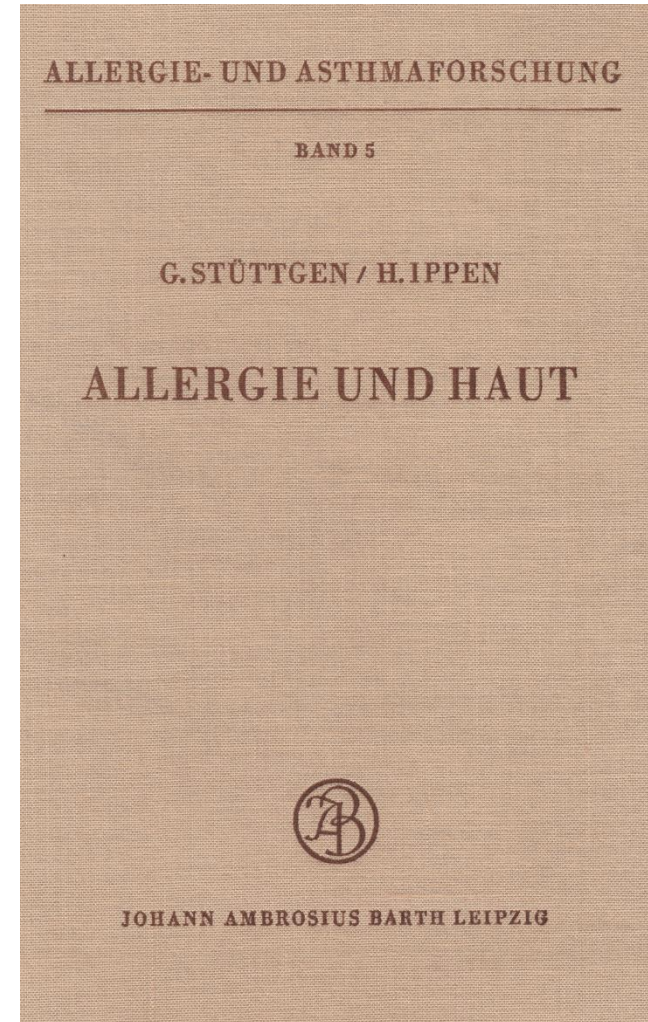
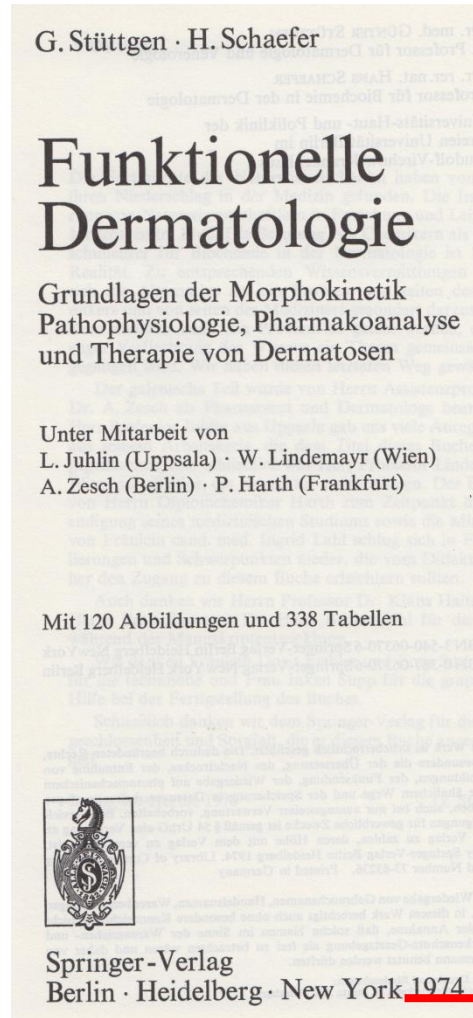
Zusammenfassung:

- Die Werbeaussage „hypoallergen“ ist eine Werbung mit Selbstverständlichkeiten. Kein kosmetisches Mittel sollte sensibilisierend wirken. Diese Prüfung ist Bestandteil der Sicherheitsbewertung eines Produktes.
- Der HRIPT ist nicht geeignet, um Sensibilisierungen bei längerem Gebrauch von Produkten sicher auszuschließen.
- Der HRIPT ist ethisch nicht vertretbar, da er irreversible Sensibilisierungen und allergische Reaktionen der Testpersonen in Kauf nimmt.
- Der Claim „hypoallergen“ ist irreführend für den Verbraucher, da er ihn so versteht, dass durch das kosmetische Mittel keine Allergien ausgelöst werden können.
- Auch Allergiker werden getäuscht, da sie sich möglicherweise in Sicherheit wiegen und bei Kauf eines kosmetischen Mittels nicht sorgfältig auf die Deklaration der Inhaltsstoffe achten.
- Im Einklang mit der Opinion des SCCNFP (XXIV/1895/98) sollte die Werbeaussage „hypoallergen“ für kosmetische Mittel nicht verwendet werden.

1968: Beginn der KoKo



1968



1969

First Evidence Showing that Langerhans Cells Are Involved in Contact Allergic Reactions in Human Skin

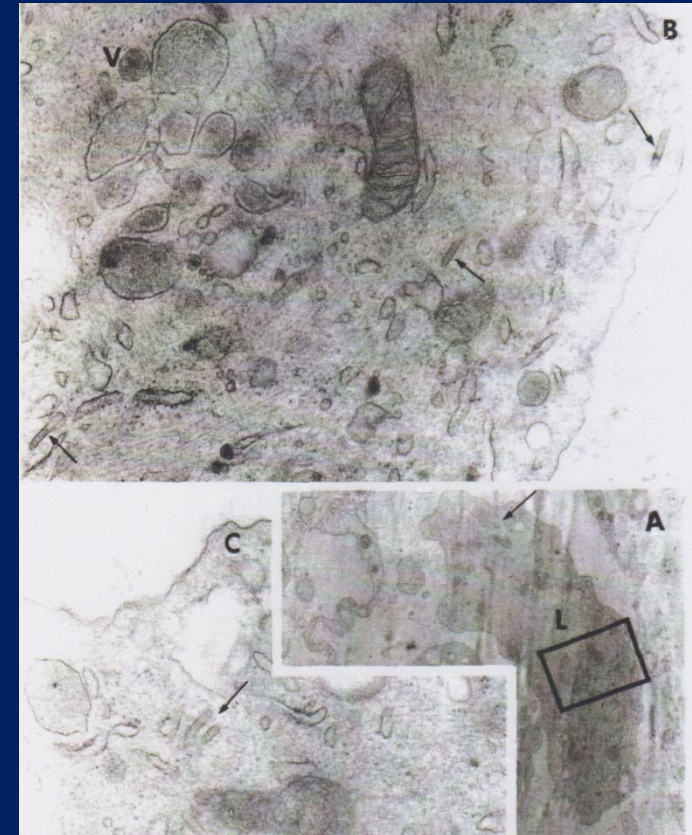
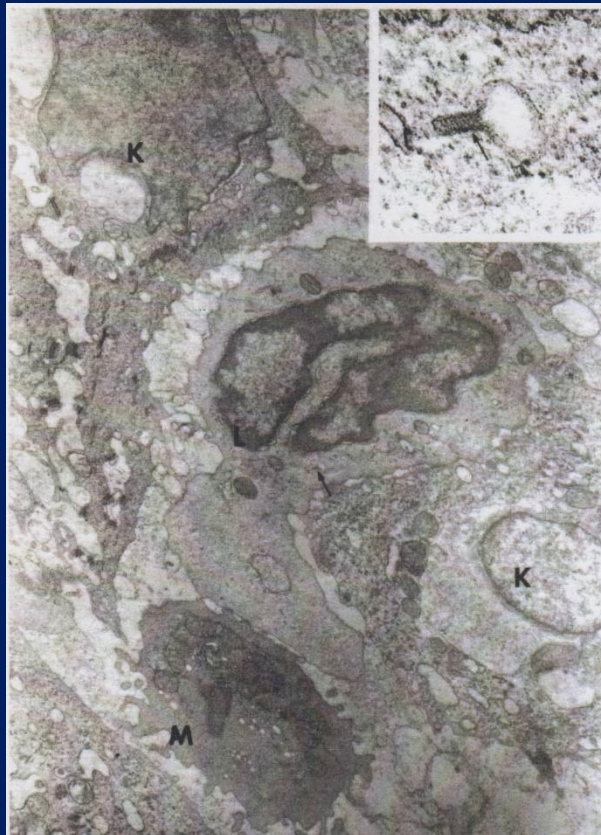
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 66:210-217, 1976
Copyright © 1976 by The Williams & Wilkins Co.

Vol. 66, No. 4
Printed in U.S.A.

THE ROLE OF LANGERHANS CELLS IN ALLERGIC CONTACT HYPERSENSITIVITY. A REVIEW OF FINDINGS IN MAN AND GUINEA PIGS*

INGA SILBERBERG, M.D., RUDOLF L. BAER, M.D., AND STANLEY A. ROSENTHAL, Ph.D.

Department of Dermatology, New York University School of Medicine, New York, New York, U. S. A.



The skin as a metabolizing organ of pro-haptens

Understanding of the fate of reactive chemicals in the skin

STUDIES ON THE SENSITIZATION OF ANIMALS WITH SIMPLE CHEMICAL COMPOUNDS*

By K. LANDSTEINER, M.D., AND JOHN JACOBS, M.D.

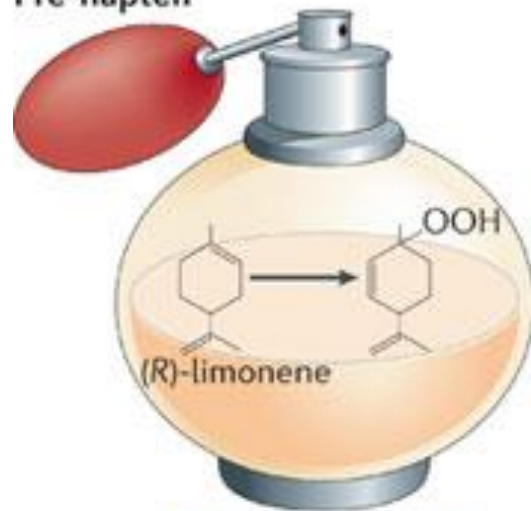
(From the Laboratories of The Rockefeller Institute for Medical Research)

PLATE 30

(Received for publication, January 25, 1935)

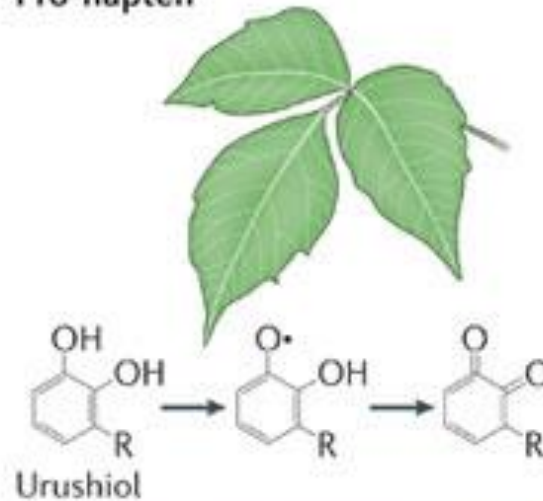


Pre-hapten



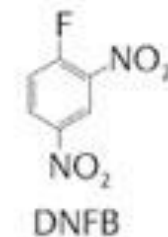
Pre-haptens oxidize before skin contact

Pro-hapten



Pro-haptens are oxidized by the host after contact

Hapten



Complete haptens are directly reactive

Cytochrom P450: Haut

STUDIES IN PORPHYRIA

VI. Biosynthesis of Porphyrins in Mammalian Skin and in the Skin of Porphyric Patients

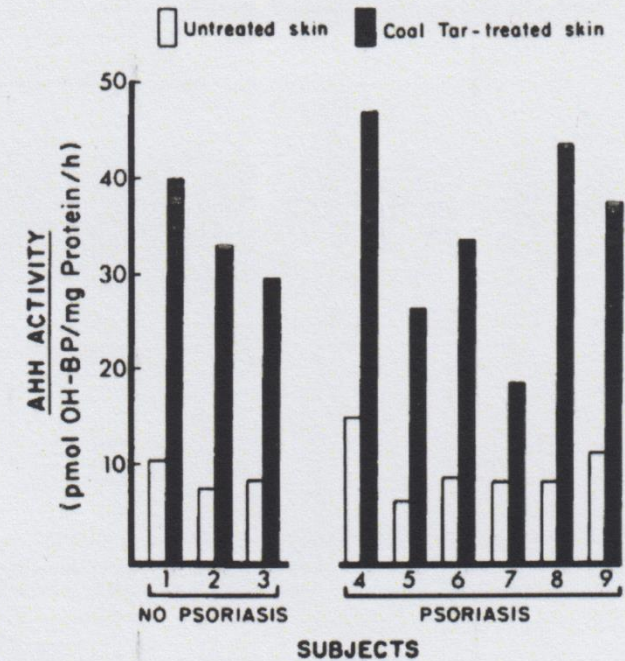
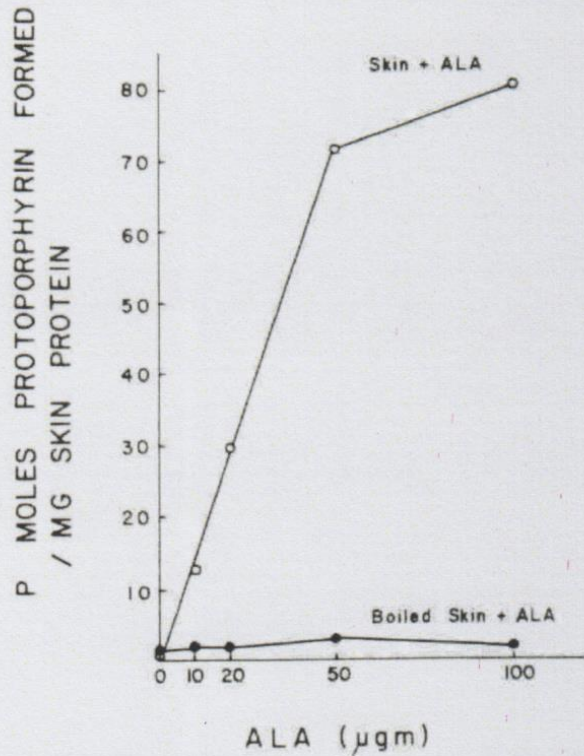
DAVID R. BICKERS, M.D., LOUISE KEOGH, B.S., ARLEEN B. RIPKIND, M.D., LEONARD C. HARBER, M.D.,
AND ATTALLAH KAPPAS, M.D.

Department of Dermatology, College of Physicians and Surgeons, Columbia University and the Rockefeller University, New York, New York, U. S. A.

Human Skin Aryl Hydrocarbon Hydroxylase

INDUCTION BY COAL TAR

DAVID R. BICKERS and ATTALLAH KAPPAS, *Division of Dermatology, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106, and The Rockefeller University Hospital, New York 10021*

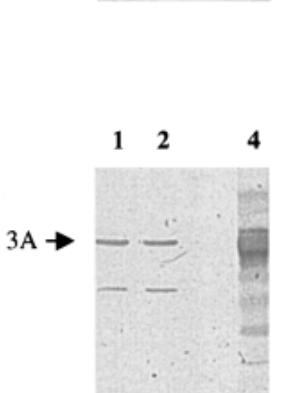
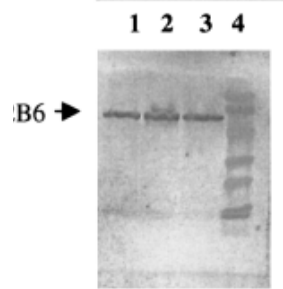
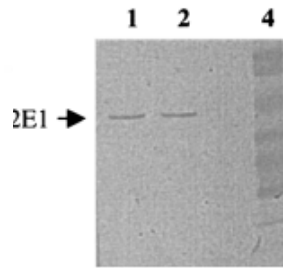
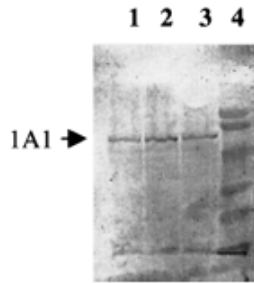
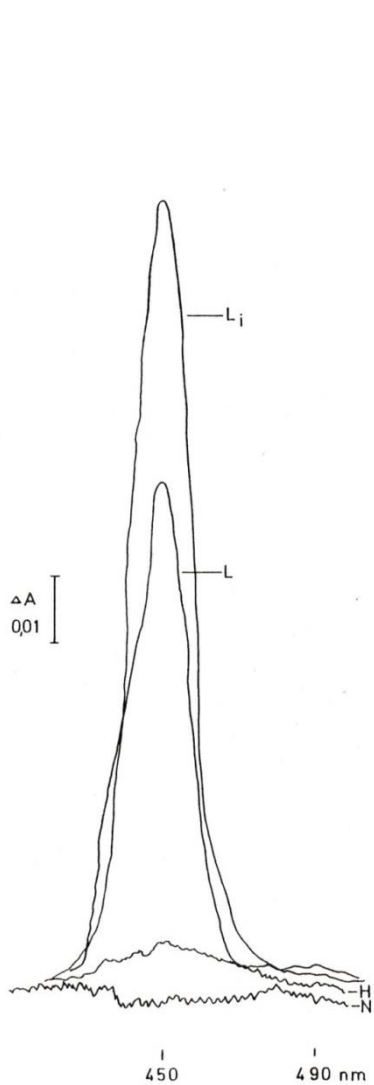


JID, 62 (1977) 5-9

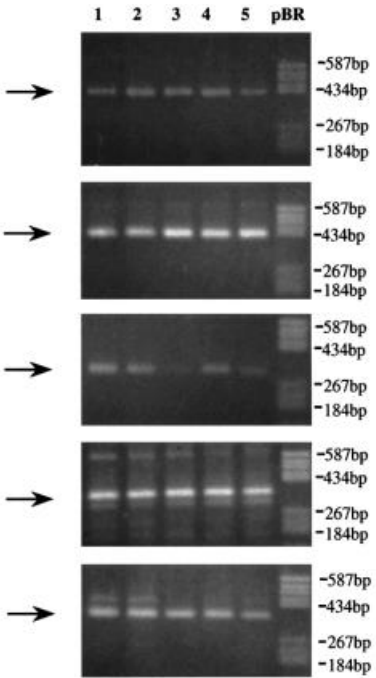
RWTH Aachen 1995

JCI, 62 (1978) 1061-1068

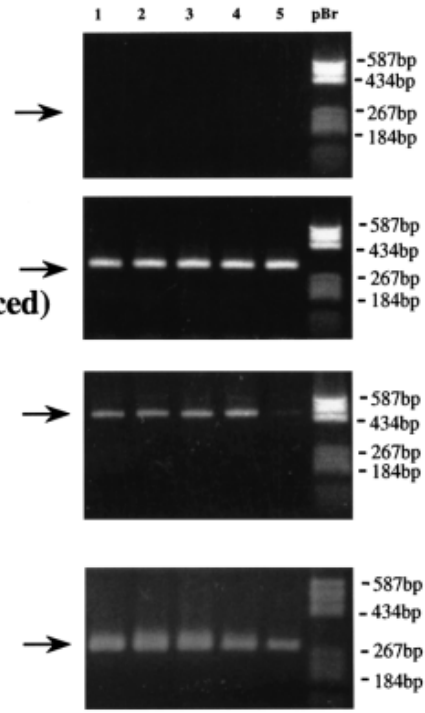
The skin as a metabolizing organ of pro-haptens



CYP1A1 (433bp)
 CYP1A1 (BA induced)
 CYP1B1 (316bp)
 CYP2B6 (377bp)
 CYP2E1 (366bp)

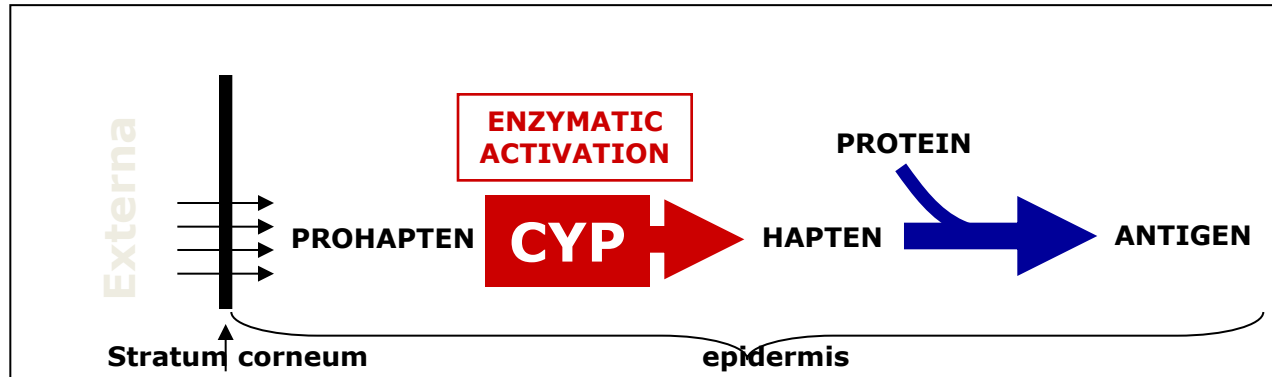


CYP3A4 (324bp)
 CYP3A4 (DEX induced)
 CYP3A5 (471bp)
 β -Actin (290bp)



Cytochrom P450

Activation of prohaptens is mediated by CYPs expressed in skin cells



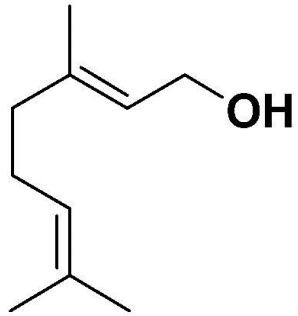
$\frac{X_2}{X_1} = 10^{\frac{n_2 - n_1}{s_2 - s_1}}$ (s = slope; n = number of PCR cycles; x = amount of CYP (pmol)) [Härtter *et al.* 2002]

	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

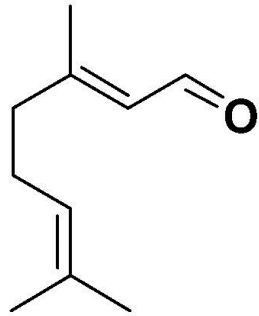


	CYP content
	(pmol)
Liver-like rhCYP cocktail	
CYP1A2	20
CYP2C9	17
CYP2C19	8.0
CYP2D6	3.0
CYP2E1	11
CYP3A4	45
Total CYP content	104

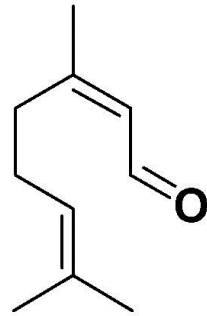
Autooxidation and CYP-metabolism important in sensitization to geraniol



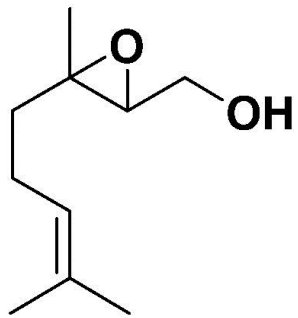
Geraniol



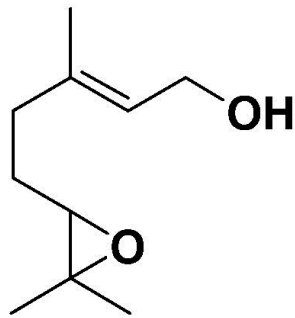
Geranial



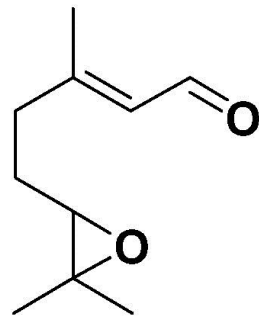
Neral



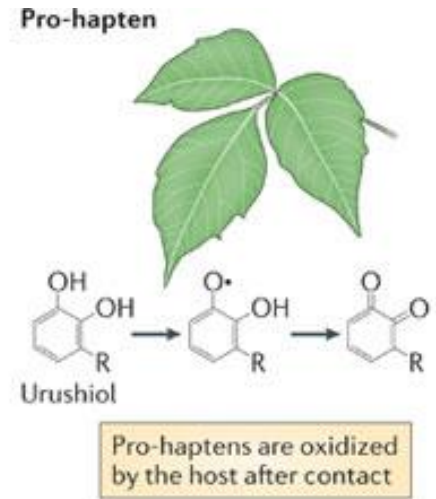
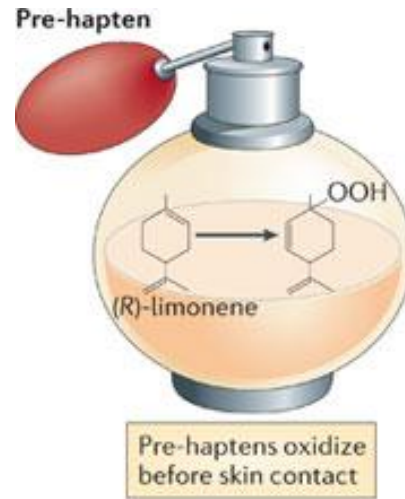
2,3-Epoxy-geraniol



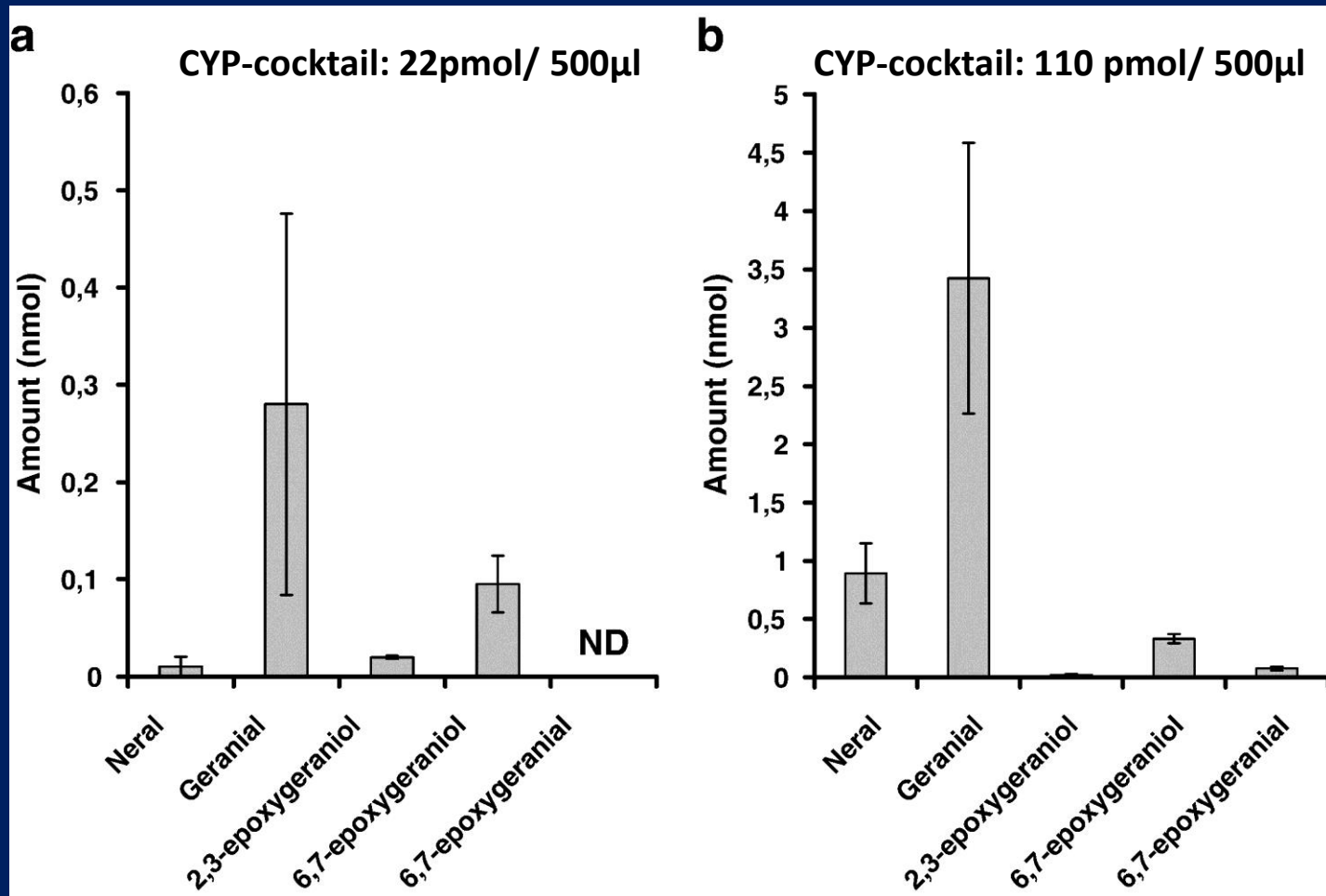
6,7-Epoxy-geraniol



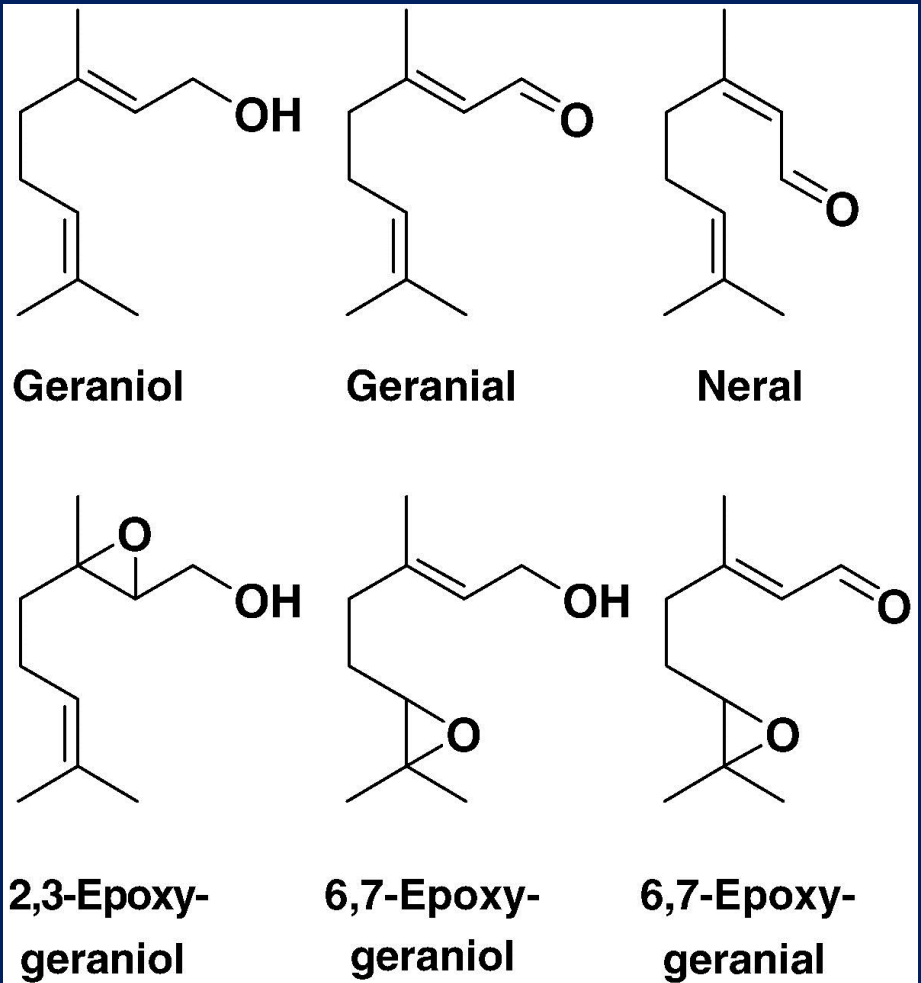
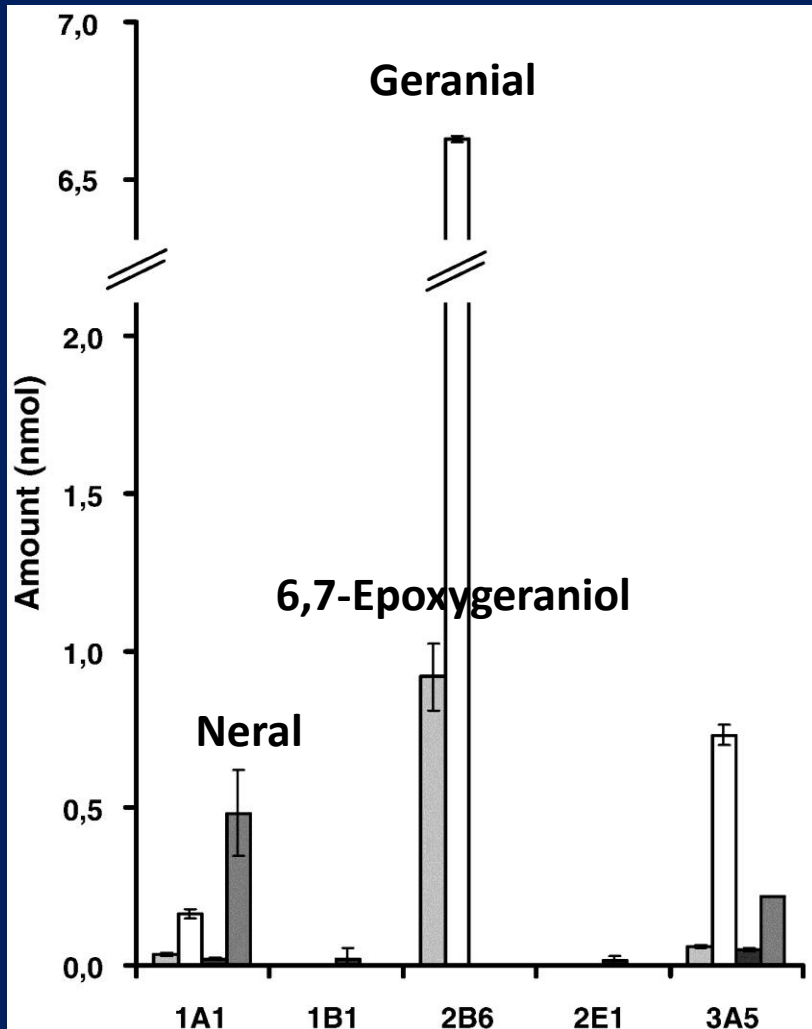
6,7-Epoxy-geranial



Autooxidation and CYP-metabolism important in sensitization to geraniol



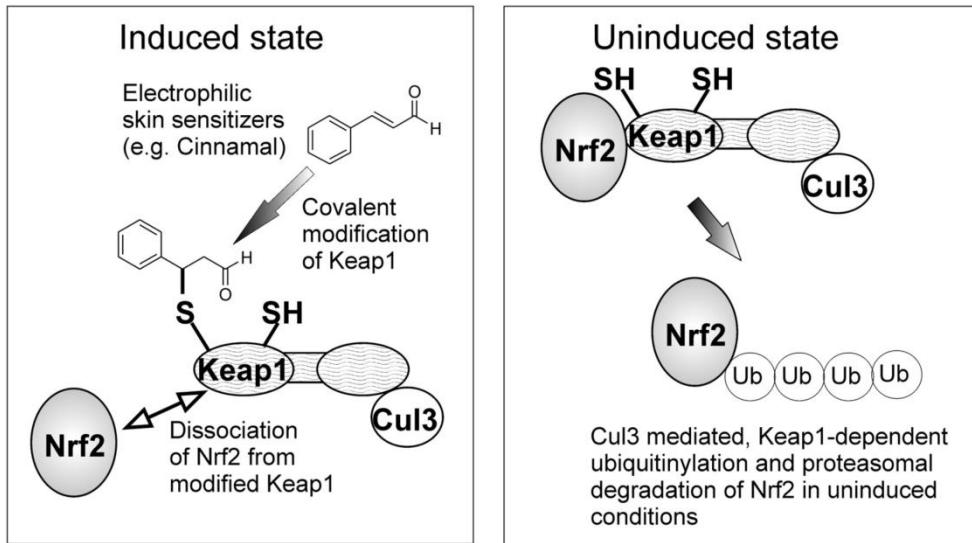
Autooxidation and CYP-metabolism important in sensitization to geraniol



Skin sensitization and Regulation

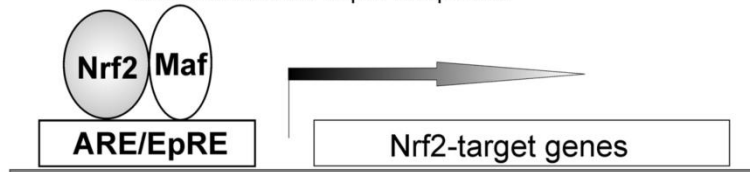
- EU cosmetic directive (EC 1223/2009)
- Registration Evaluation, Authorization, and Restriction of Chemical Substances REACH (EC 1907/2006)

The Nrf2-Keap1-ARE Toxicity Pathway as a Cellular Sensor for Skin Sensitizers



Nuclear accumulation of Nrf2 in induced state

Heterodimer of Nrf2 with small Maf binds to consensus ARE/EpRE sequence



Nrf2-dependent genes reported to be induced by skin sensitizers:

- NQO1 (quinone reductase)
- Interleukin-8
- AKR1C2 (aldo-keto reductase)
- Thioredoxin
- Thioredoxin reductase I
- HMOX1
- CES 1

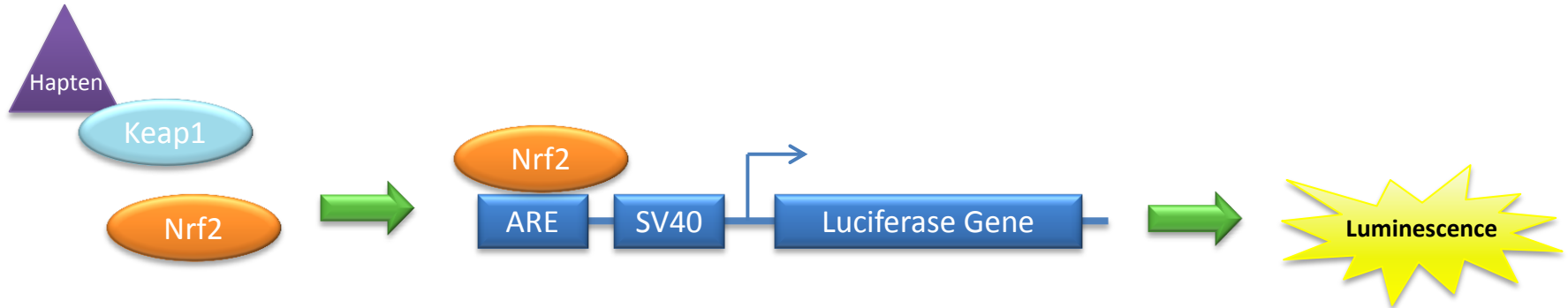
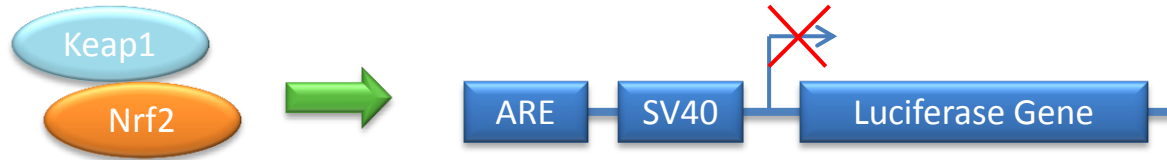
ARE: Antioxidant response element

Keap1: Kelch-like ECH-associated protein 1

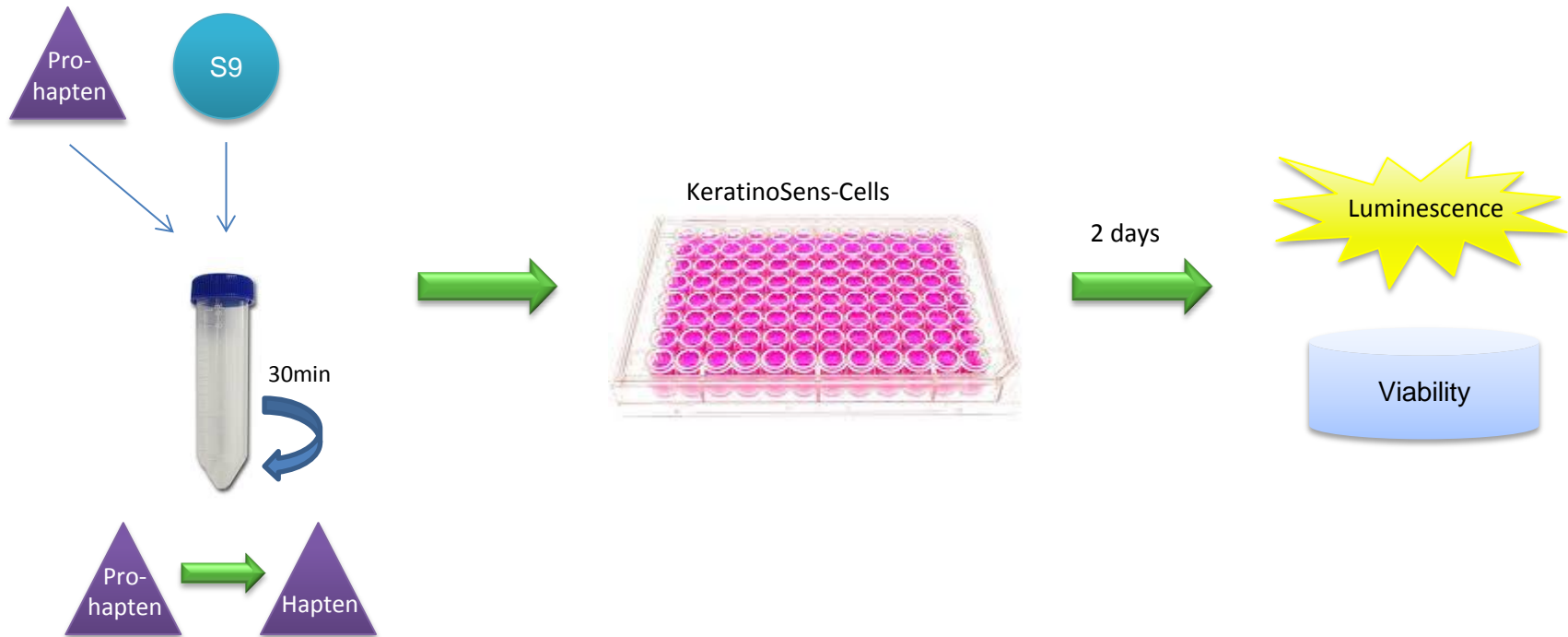
NRF2: nuclear factor-erythroid 2-related factor 2

A Natsch: Toxicological Sciences, Volume 113, 2010, 284–292

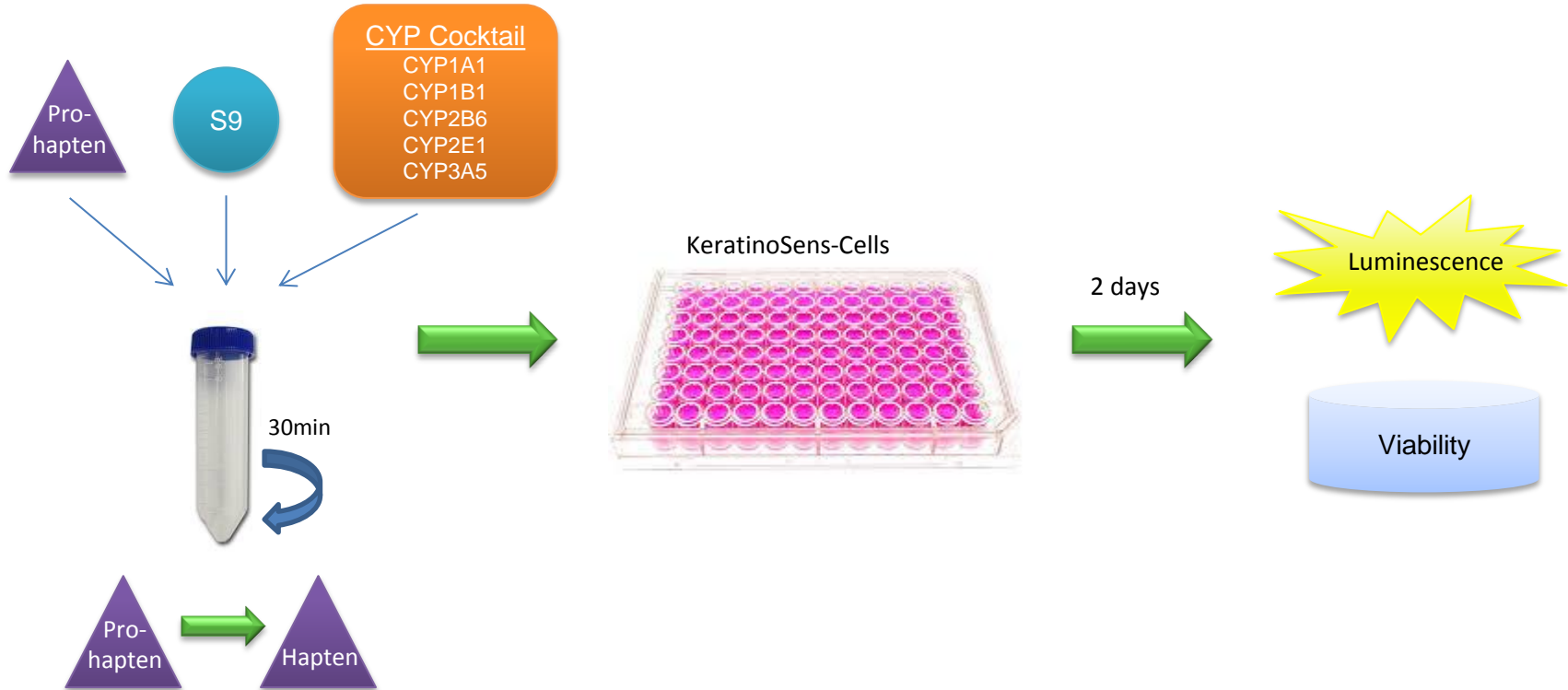
KeratinoSens[®]-Assay



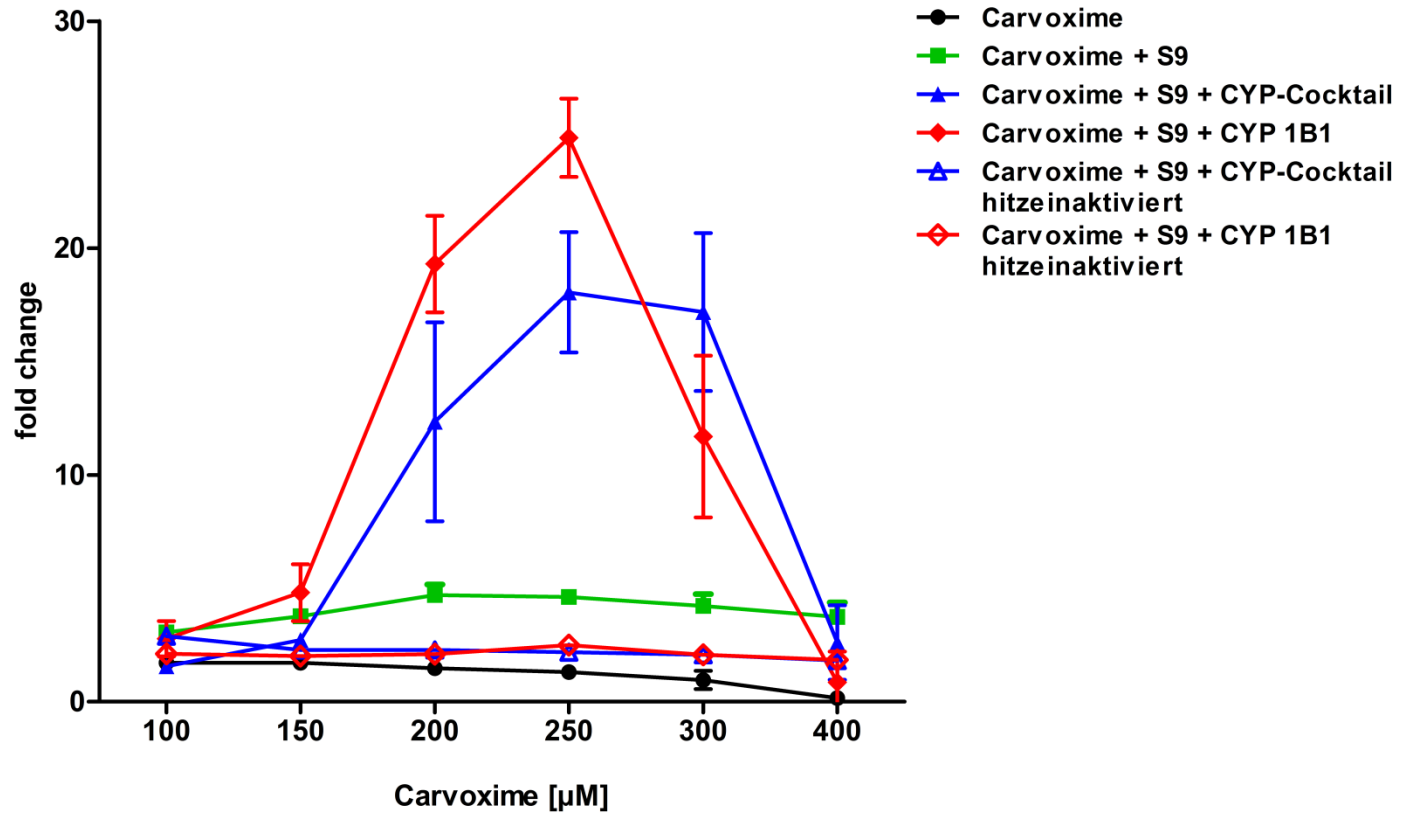
Modified KeratinoSens[®] Assay



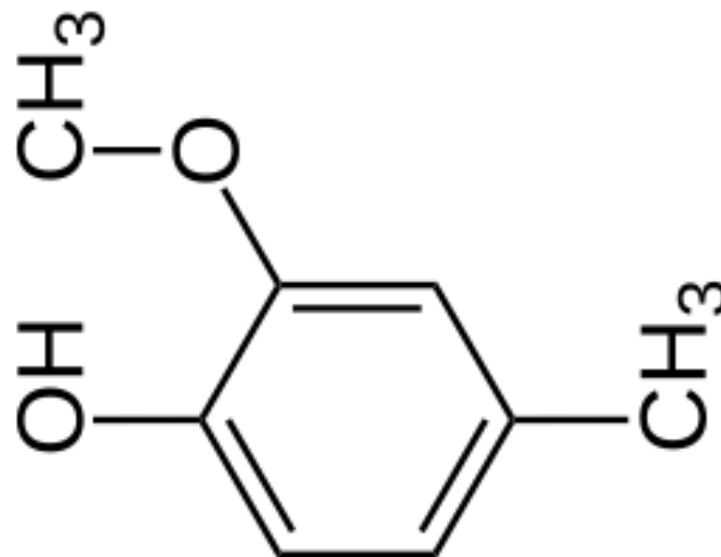
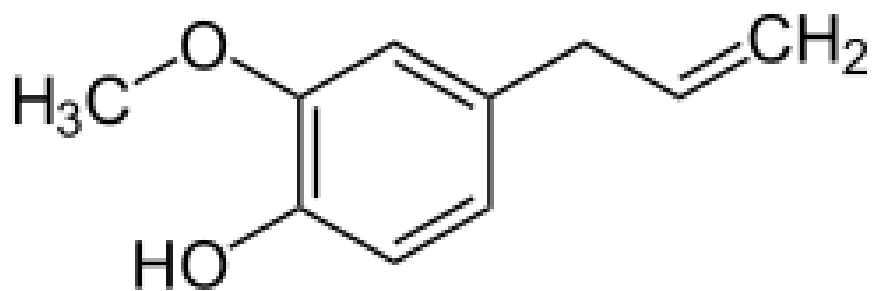
Modified KeratinoSens[®] Assay



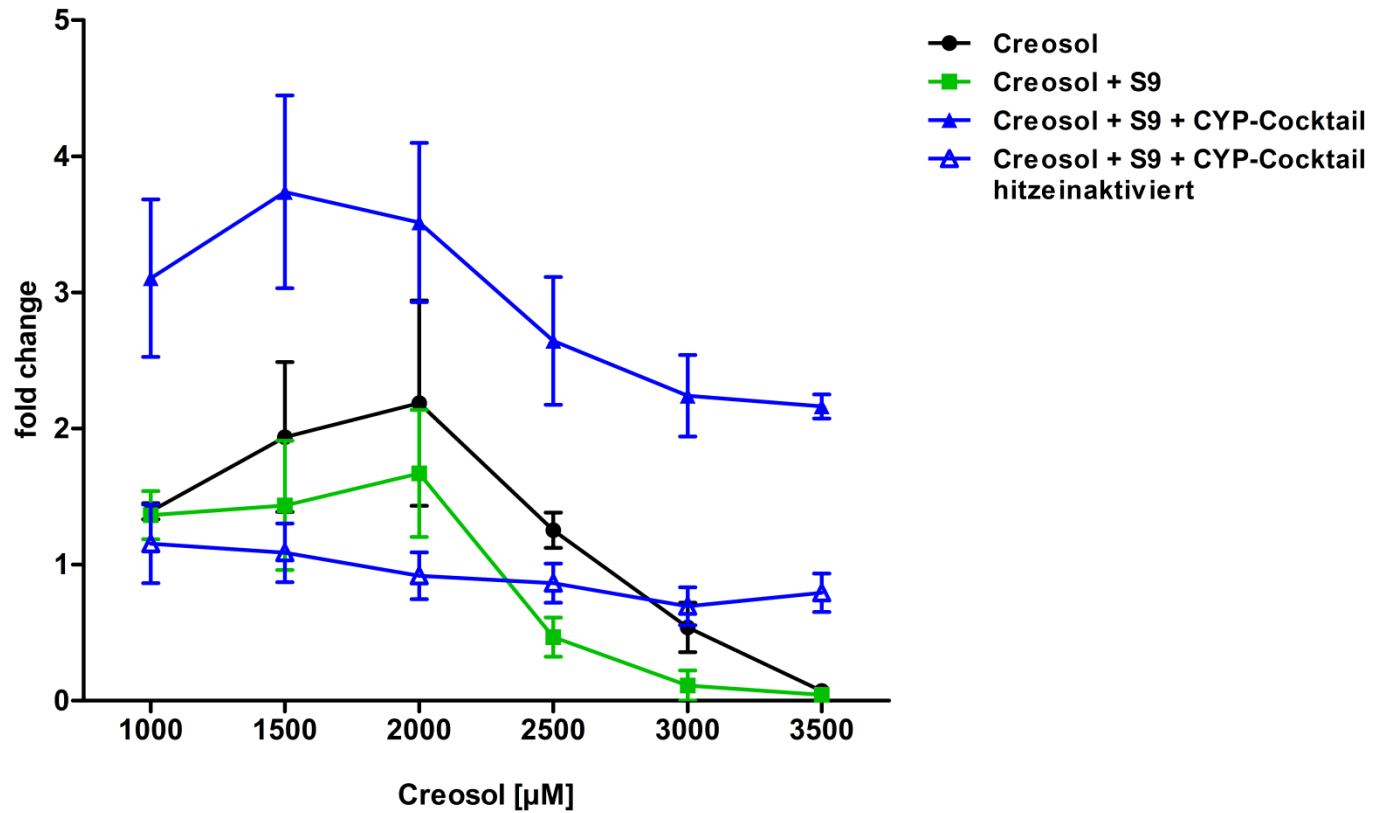
KeratinoSens with Carvoxime



Eugenol - Creosol

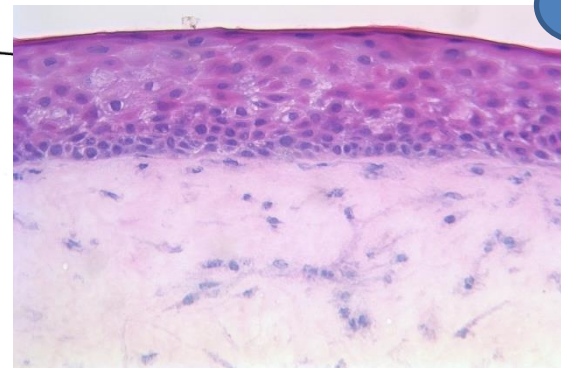
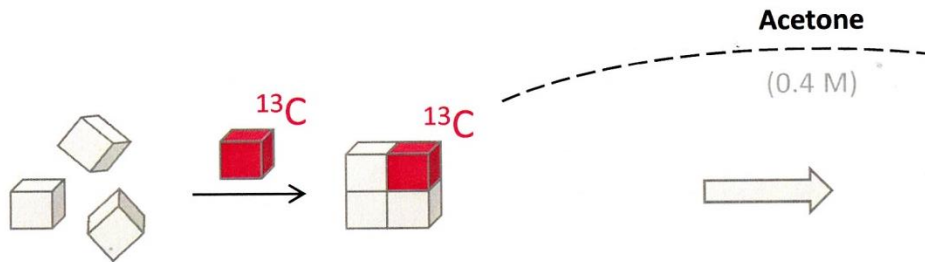


KeratinoSens mit Creosol



Methods

Experimental protocol: 4 key steps



Incubation
1 h \Rightarrow 24 h
Reaction
kinetics

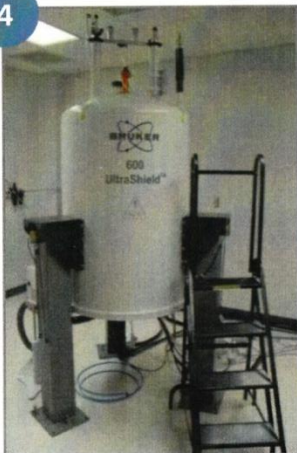
1 Synthesis of substituted allergens

^{13}C selective substitution

LABORATORY
of DERMATO-CHEMISTRY

UNIVERSITY HOSPITAL
of STRASBOURG

4

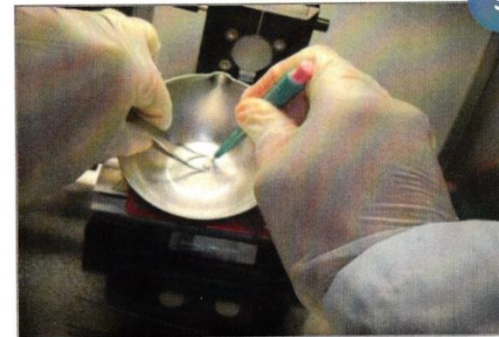


Insert + RHE + D_2O

Analysis by HR-MAS NMR

1D (^{13}C) / 2D (HSQC) experiments

3

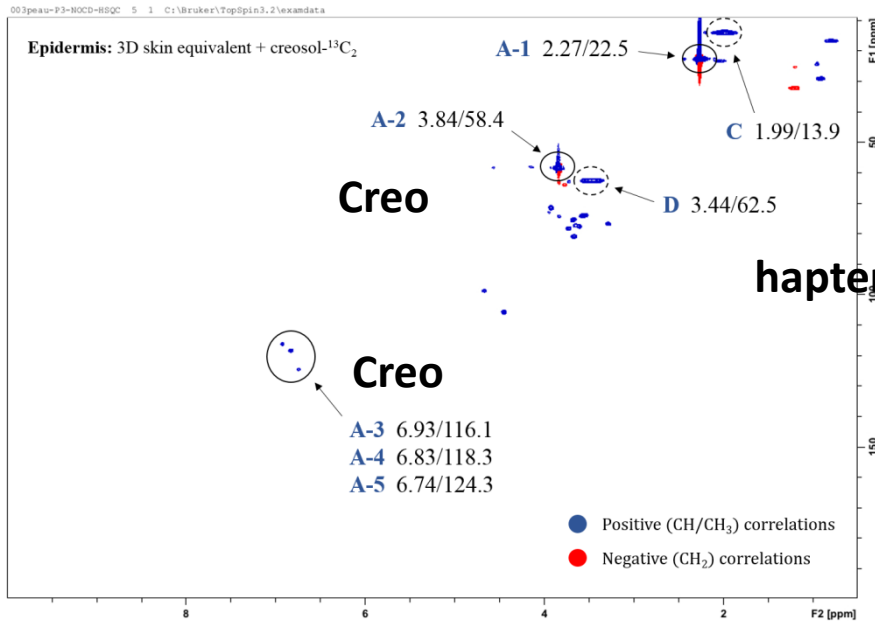


Preparation of samples ($-20\text{ }^\circ\text{C}$)



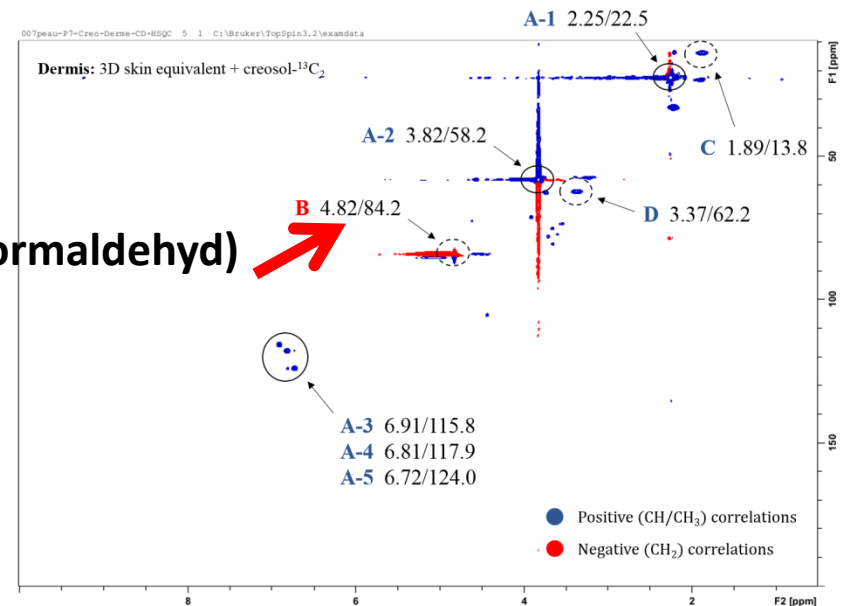
HSQC (2D) spectra of treated samples epidermal layers (left) and dermis (right)

Creo

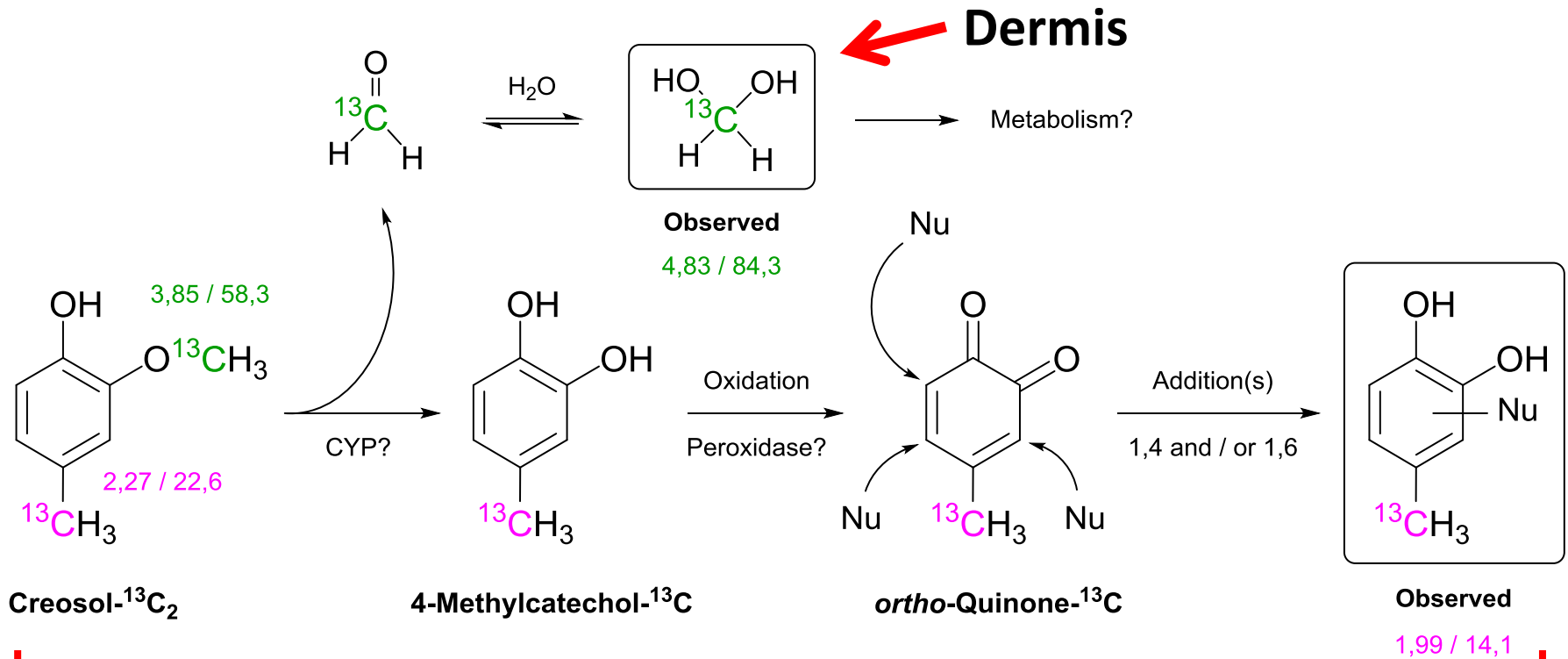


1,2 benzoquinone-hapten-protein-adduct
hapten-protein-adduct

Methandiol (monohydrated formaldehyd) 



Bioactivation/reactivity of creosol-¹³C₂ in 3D skin equivalents



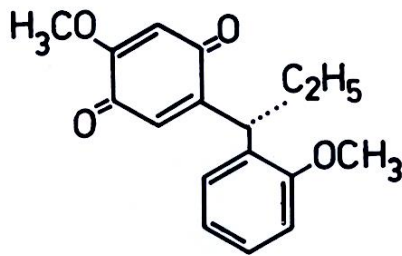
Epidermis + Dermis

Haut:

Zielorgan allergischer Reaktionen auf kleinmolekulare Substanzen



Dalbergia latifolia (Palisander)



Dihydro-2',4-dimethoxydalbergione

R-4-Methoxydalbergion 1%

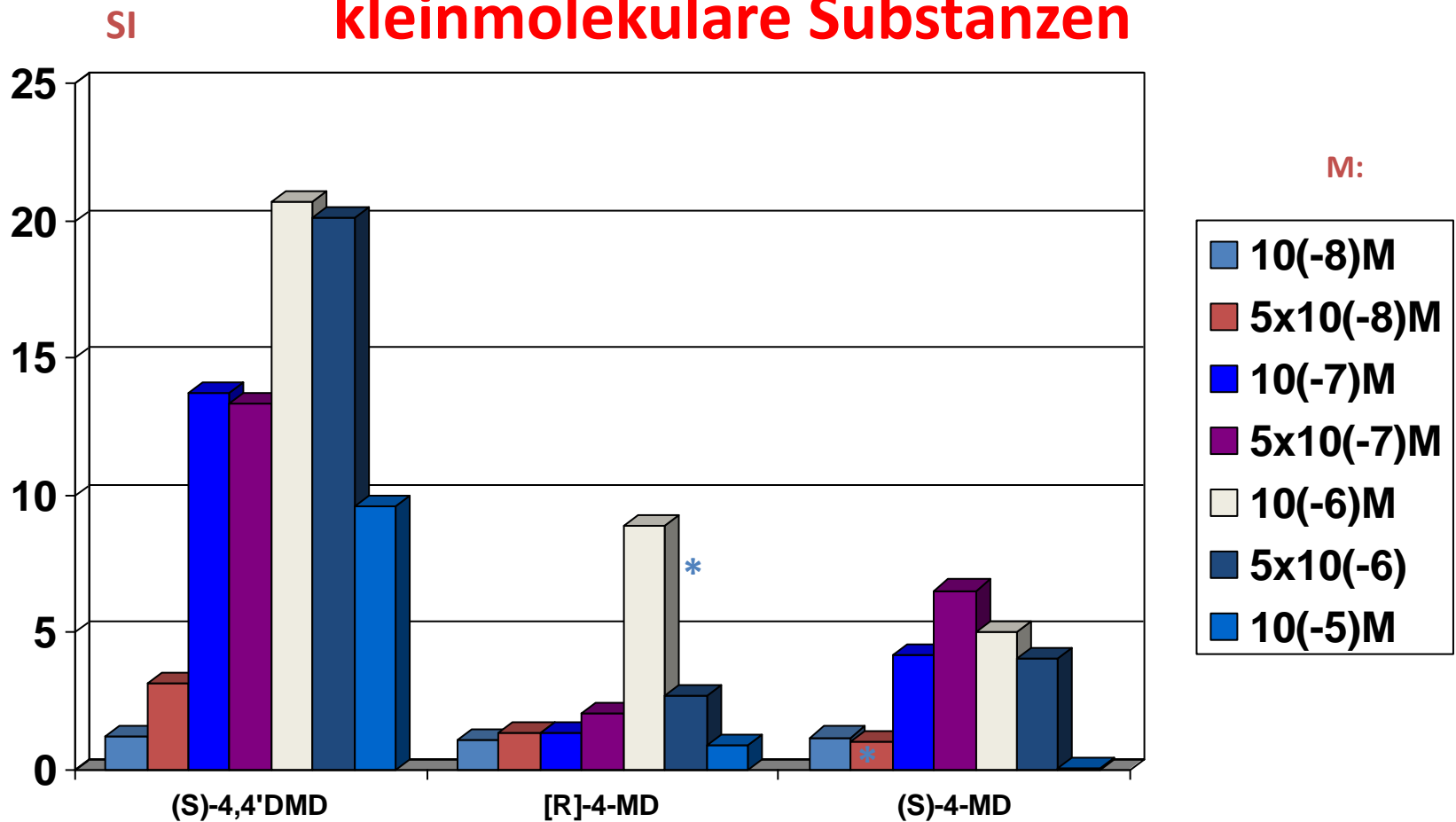
S-4-Methoxydalbergion 1%

Obtusachinon 1%

S-4,4-Dimethoxydalbergion 1%



Haut: Zielorgan allergischer Reaktionen auf kleinmolekulare Substanzen



DMD: Dimethoxydalbergion; MD: Methoxydalbergion

SI= Stimulation index <2: no increased proliferation; 2-3: prolif. questionable; >3: increased proliferation

PHA: 13,20; Tetanustoxin: 5,75
TT+solvent: 5,85; Solvent: 1,08