



What is known based on metabolism, induction and elicitation data (animal and clinical) regarding pro-hapten activation, cross-reactivity of esters and breakdown products - mechanistic understanding and quantitative follow-up...and a raccoon... (J. Prévert - Inventory)

Prof. Jean-Pierre Lepoittevin
University of Strasbourg, France



IDEA Workshop
Pre- and pro-haptens
Leuven, 20th-21st October 2015





Specificity of contact sensitization...

- **By definition and experience contact sensitization is “chemical” specific...**
- **This specificity is associated to a supramolecular association between MHC/antigenic peptide/TCR molecules...**
- **Inter-individual variations should be expected...**





Cross-reactions at a molecular level...

- **Lack of specificity in antigenic peptide - TCR interactions...**
- **Selection of T-cell clones during the sensitization process...**
- **Activation of T-cell clones during the elicitation process...**
- **Inter-individual variations should be expected...**



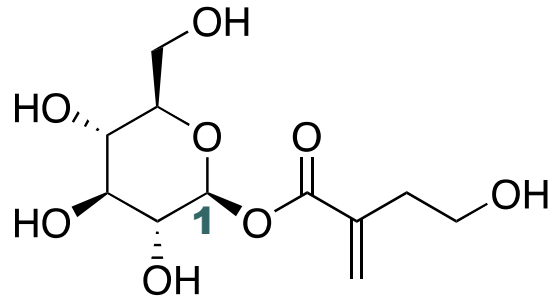


True cross-reaction between two sensitizers A and B...

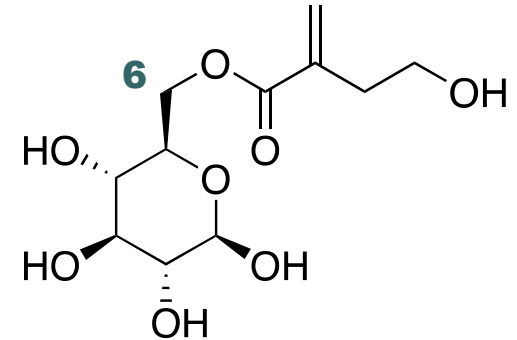
- **A and B are chemically different**
- **A is not metabolized into B...**
- **B is not metabolized into A...**
- **A is not transformed into B...**
- **B is not transformed into A...**



False cross-reaction between two sensitizers A and B...

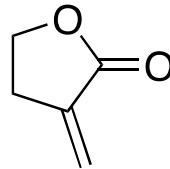
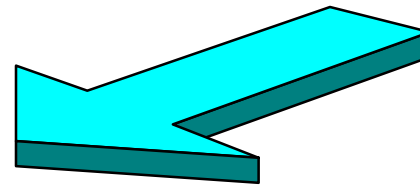
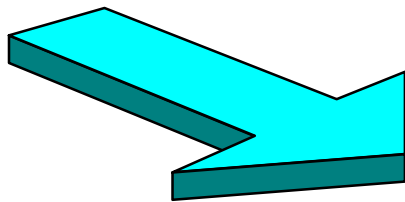


1-Tuliposide A



6-Tuliposide A

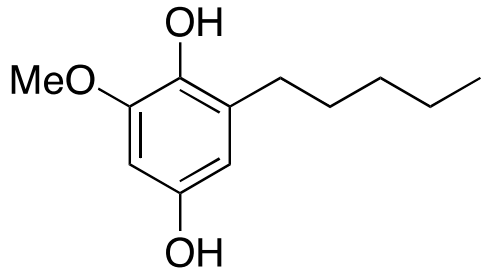
hydrolysis



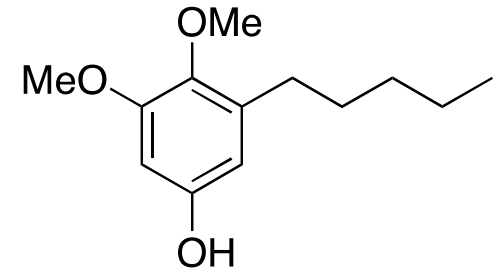
Tulipalin A



False cross-reaction between two sensitizers A and B...

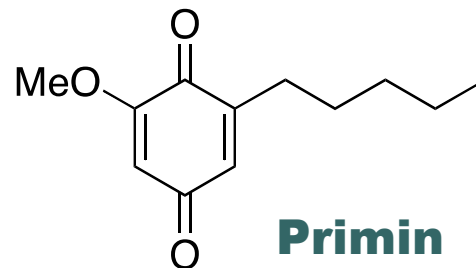
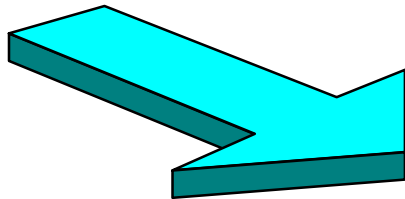


Miconidin

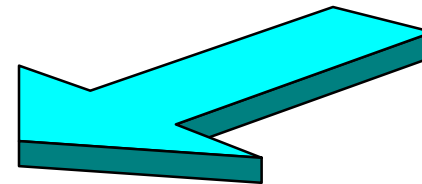


Miconidin methyl ether

metabolism



Primin
(Primula obconica L.)





How to investigate cross-reactions...

- **Using animal models...**
- **At a cellular level...**
- **In Human individuals...**
- **...**





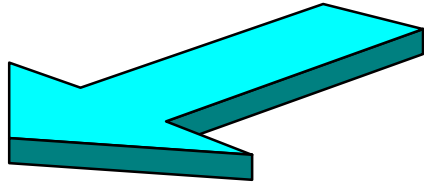
How to investigate cross-reactions...

- **Using animal models...**
- **At a cellular level...**
- **In Human individuals...**
- **...**

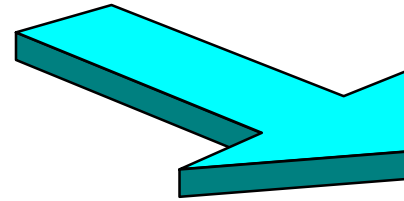




Animal models (Guinea-Pigs)



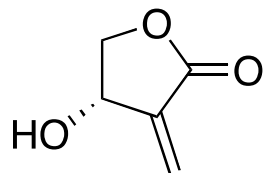
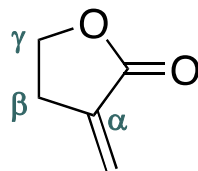
Functional analogy...



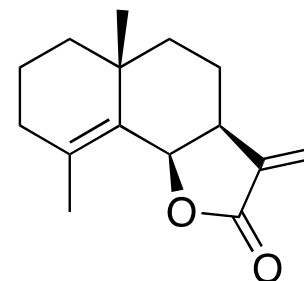
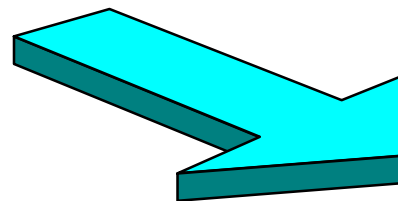
Structural analogy...



α -Methylene- γ -butyrolactones...



Tulipalin B
(*Tulipa gesneriana* L.)

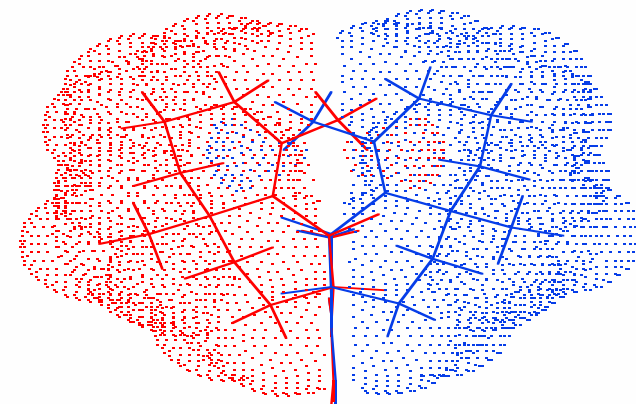
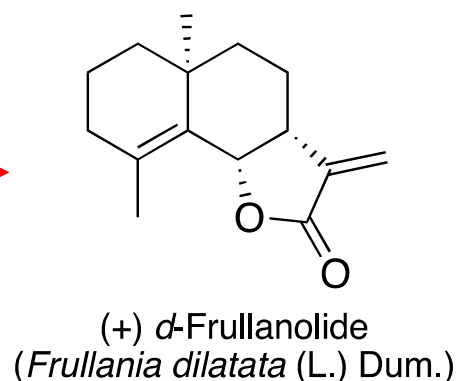
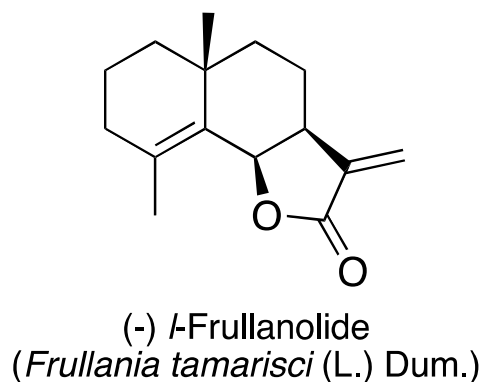


(-)-Frullanolide
(*Frullania tamarisci* L.)



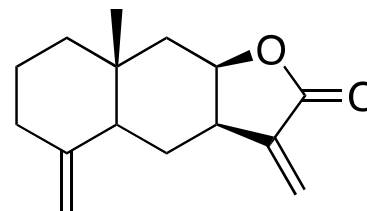
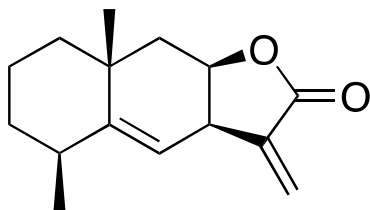
Lepoittevin et al. Chem Rec 2009; 9; 258-270

α -Methylene- γ -butyrolactones...

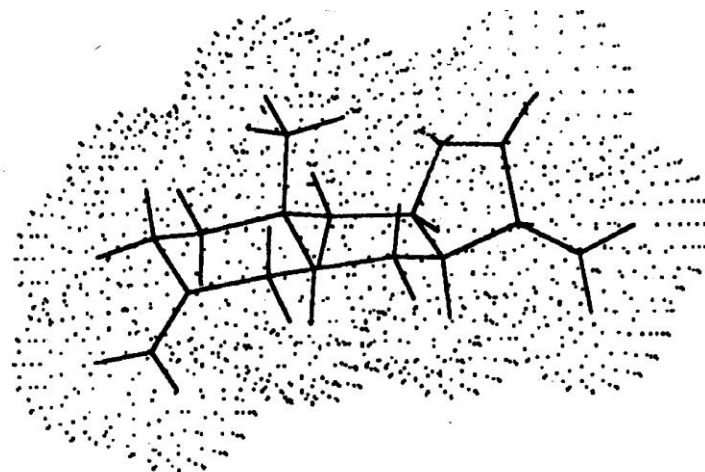
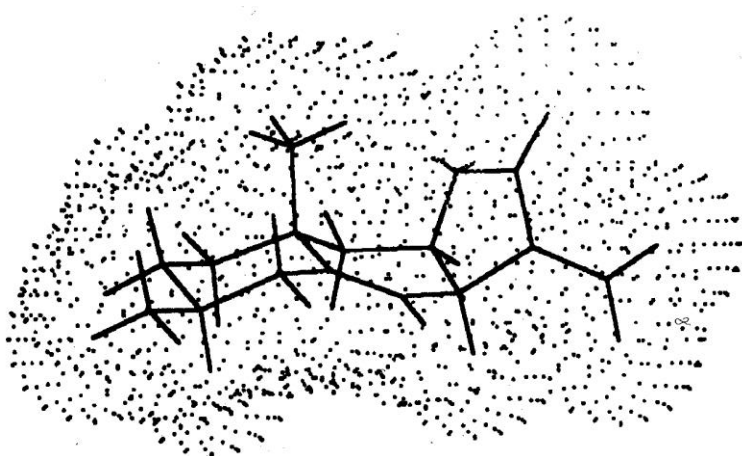


Enantiomers have similar « chemical » structures but very different « stereochemical » structure...

True cross-reaction between two sensitizers A and B...



Alantolactone
(Inula helenium L.)





How to investigate cross-reactions...

- Using animal models...
- **At a cellular level...**
- In Human individuals...
- ...

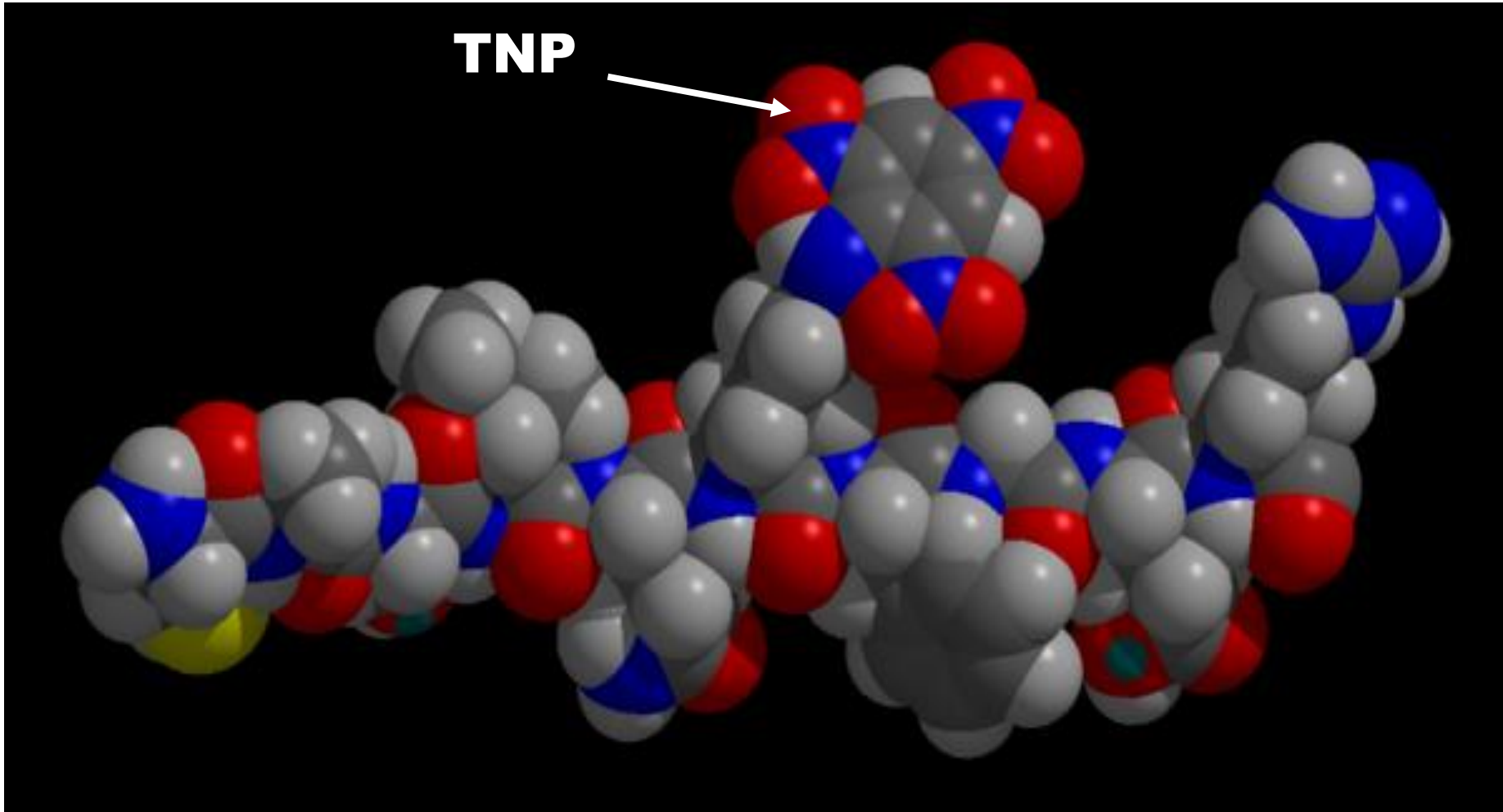




At a cellular level...

- **Sensitization of mice with TNP-chloride...**
- **Isolation of T-cell clones reactive to TNP-sulfonate...**
- **Screening with proteins and peptides modified by TNP...**
- **Identification of lysine-TNP modified peptides...**

● ● ● | **At a cellular level...**





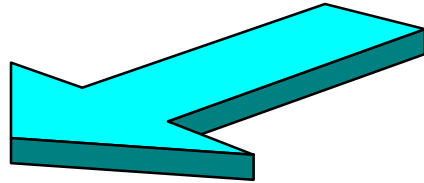
How to investigate cross-reactions...

- Using animal models...
- At a cellular level...
- **In Human individuals...**
- ...

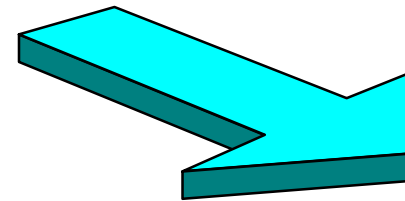




Clinical studies



Cross-reactions...

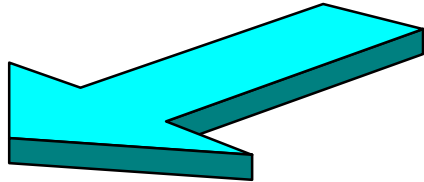


Co-sensitization...

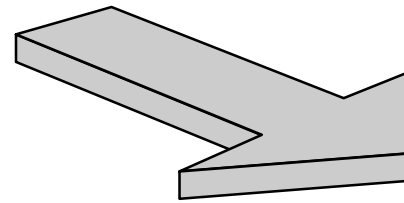




Human individuals...



**Re-test
methodology...**



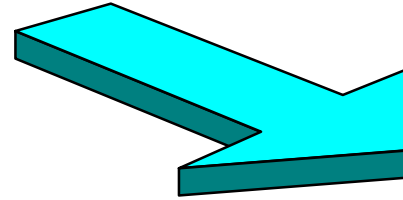
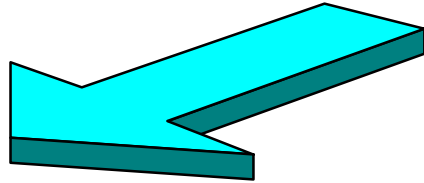
**Statistical analysis
of clinical data...**



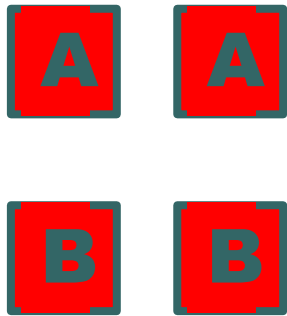
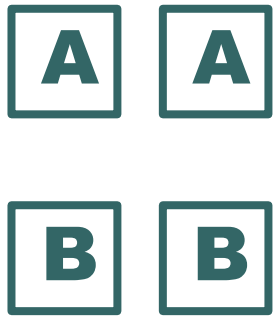
Human individuals...



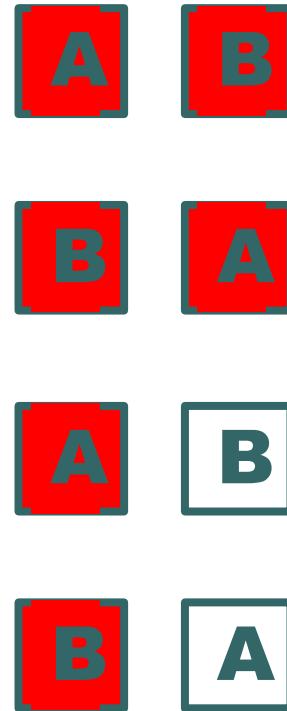
1st test



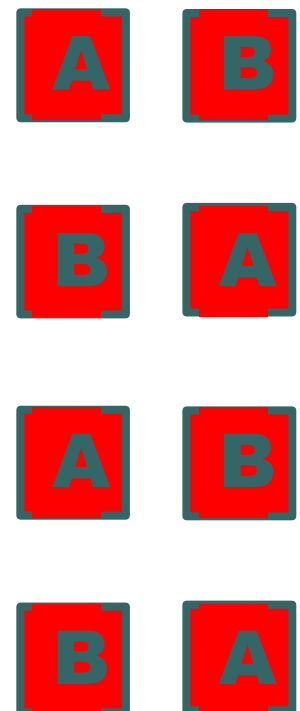
2nd test



24h



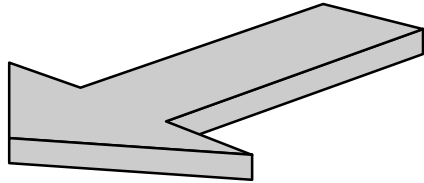
4h



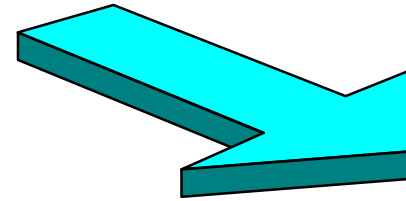
24h



Human individuals...



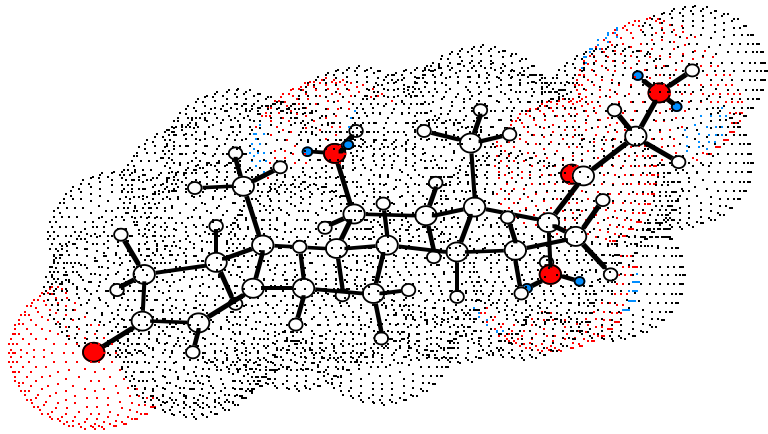
**Re-test
methodology...**



**Statistical analysis
of clinical data...**



Multiple positive tests to corticosteroids



Hydrocortisone





Cross-reactions among corticosteroids

Group A : Hydrocortisone type

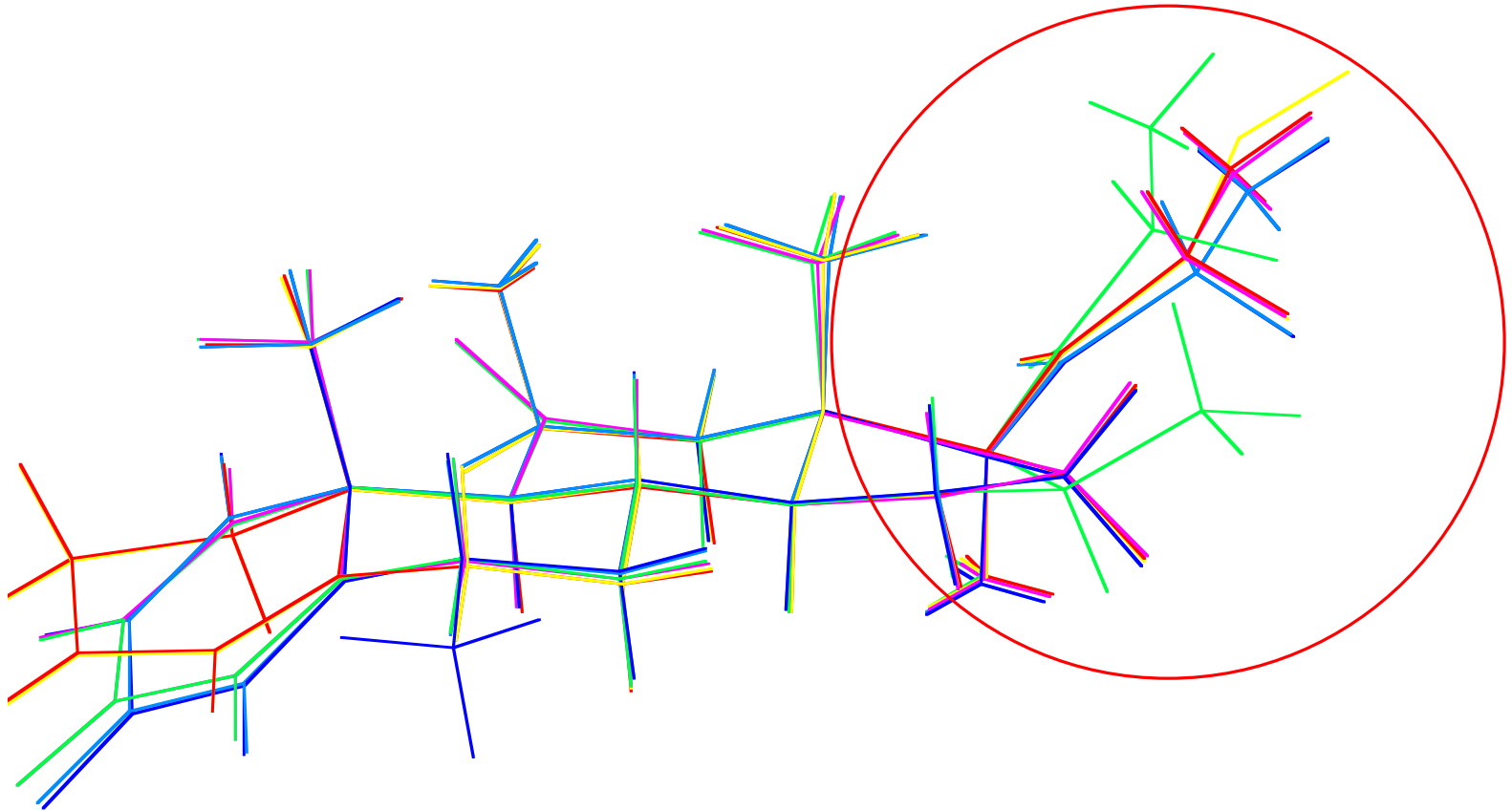
Group B : Triamcinolone acetonide type

Group C : Betamethasone type

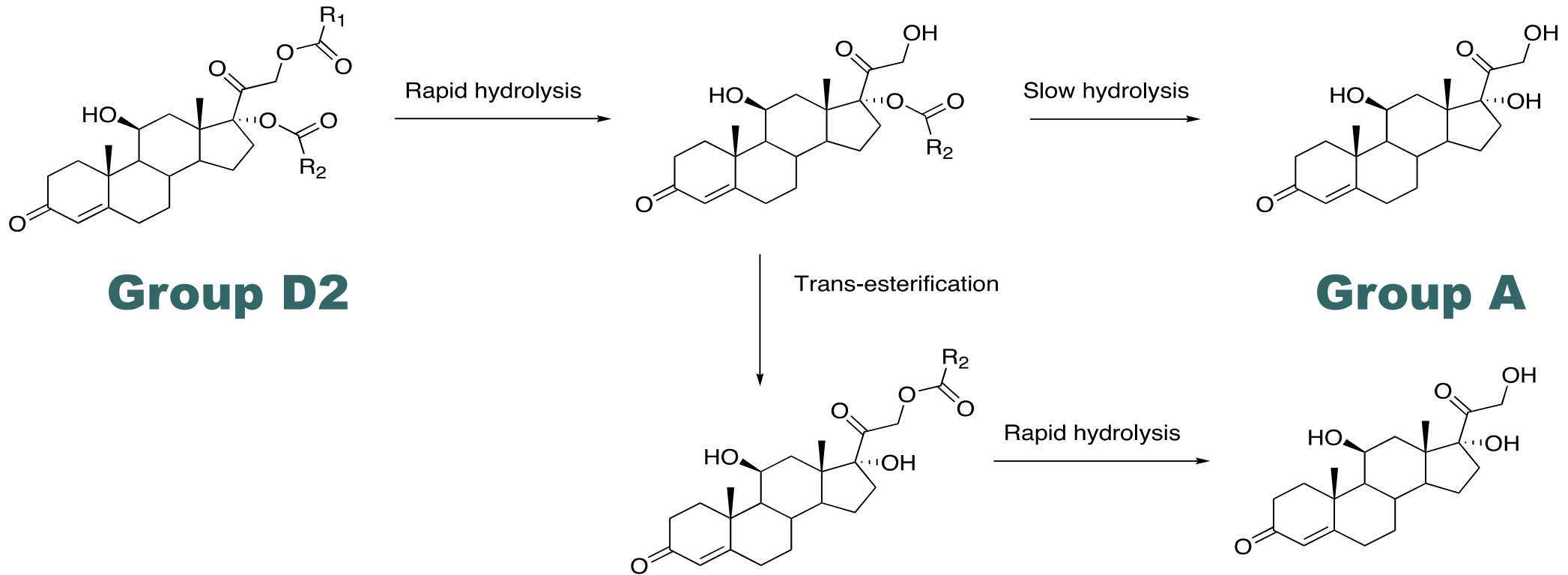
**Group D : Hydrocortisone-17-butyrate type
(D1 and D2 subdivision)**



Group A

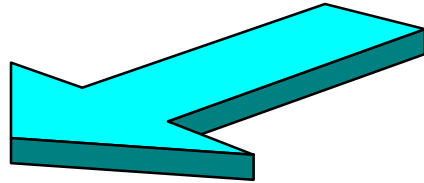


Hydrolysis of esters...

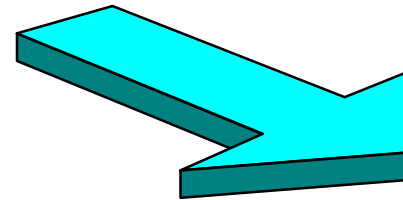




Prohaptens ?



**Modified by skin
metabolism...**



**Reactive
metabolites...**



Metabolism of xenobiotics

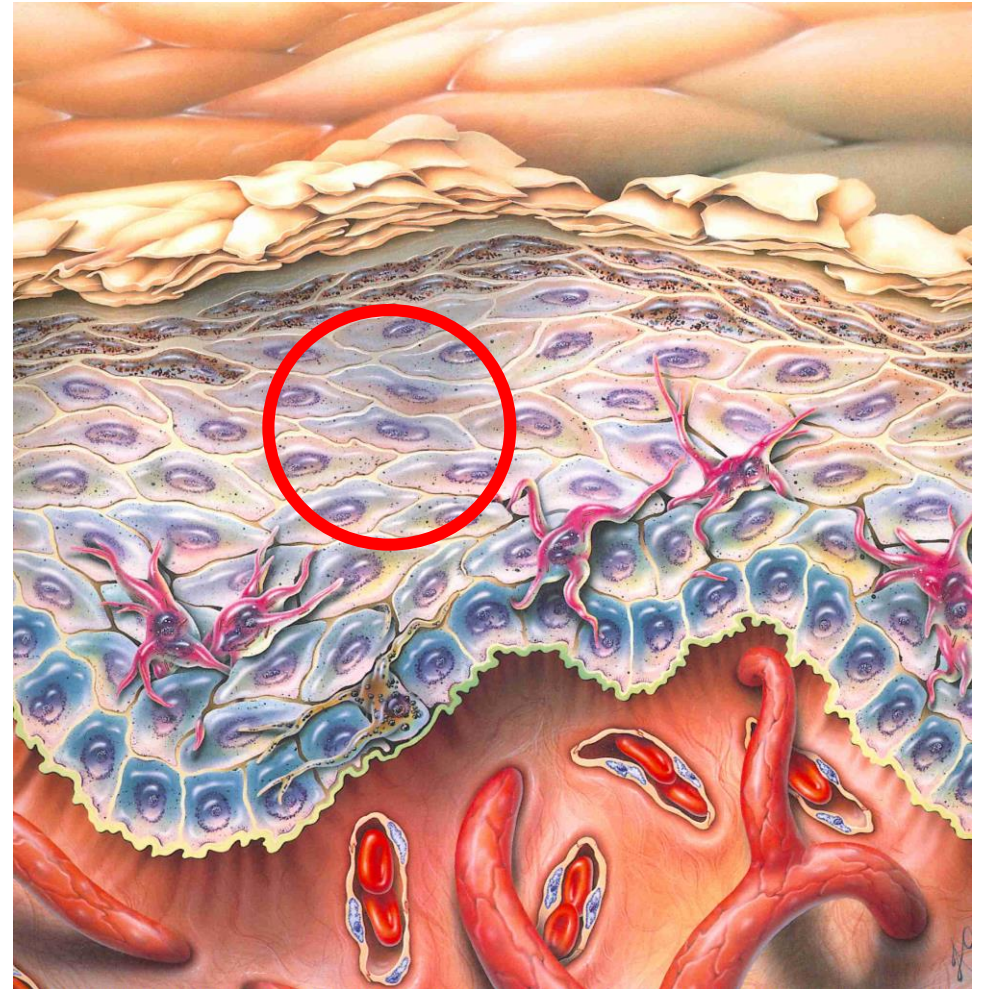
Main enzymatic systems identified in Human epidermis...

EC 1	Oxydoreductases	Cytochromes P450 (CYPs)	1A1/1B1, 2B6/2E1, 3A5/3A7...
		Alcohol / Aldehyde deshydrogenases (ADH / ALDH)	EC 1.1.1.1 / EC 1.2.1.3
		Peroxisomes	EC 1.11.x
EC 2	Transferases	Catechol-O-methyl transferases (COMT)	EC 2.1.1.6
		N-acetyltransferases (NAT)	EC 2.3.1
		Glucuronosyltransferases (UGT)	EC 2.4.1.17
		Glutathion S-transferases (GST)	EC 2.5.1.18
		Sulfotransferases (SULT)	EC 2.8.2.x
EC 3	Hydrolases	Esterases (ES)	EC 3.1.x



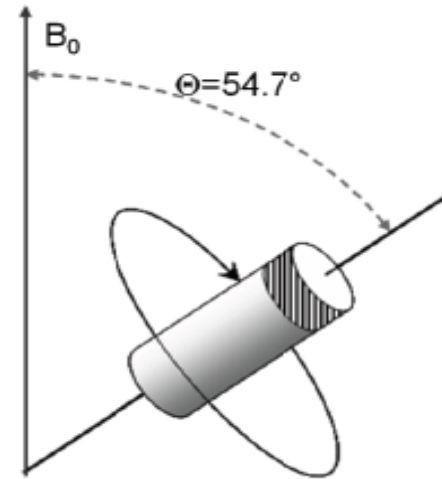
● ● ● | Non invasive approach...

- **Direct observation...**
- **Highly non homogeneous environment...**

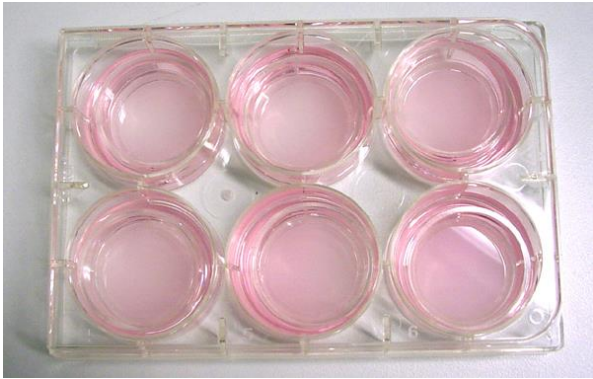


HRMAS NMR...

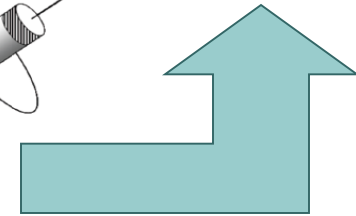
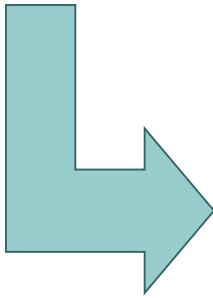
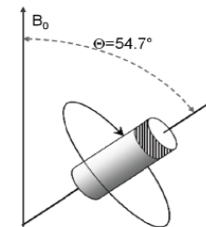
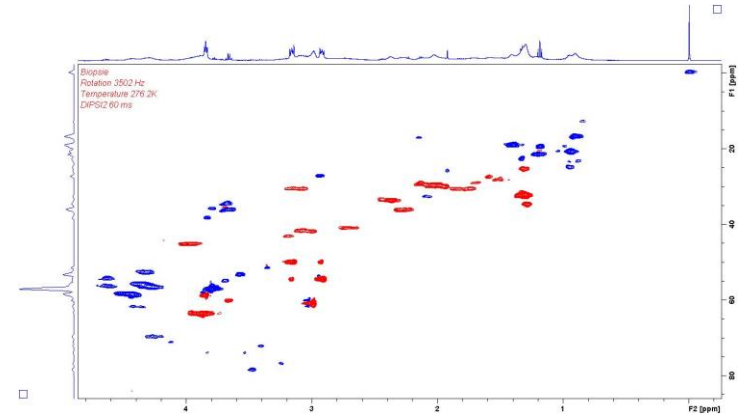
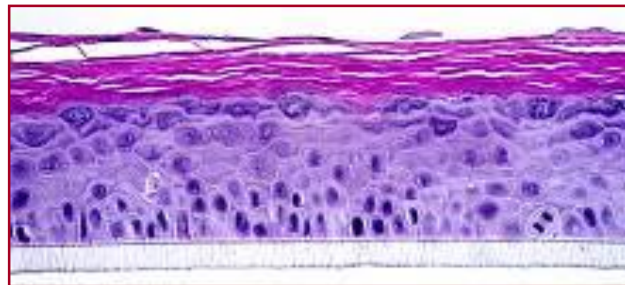
- **High-Resolution Magic Angle Spinning “HRMAS” Nuclear Magnetic Resonance...**
- **Bring to zero inhomogeneity associated with the sample...**



HRMAS NMR...



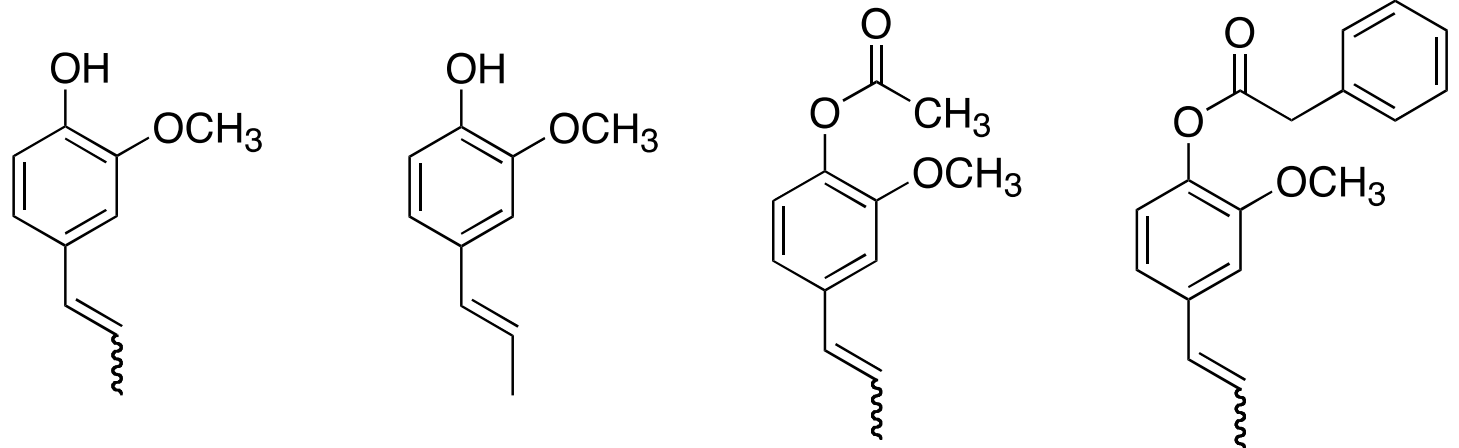
**Test
Chemical**



Fragrance substitution...



Isoeugenol can be substituted by derivatives transformed back into isoeugenol... by abiotic or biotic pathways ?



White I., et al. Contact Dermatitis 1999, 41, 272-275



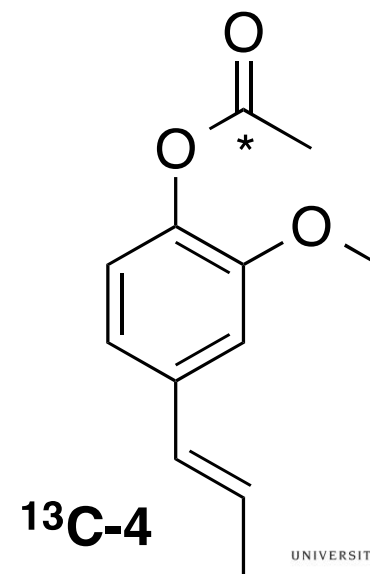
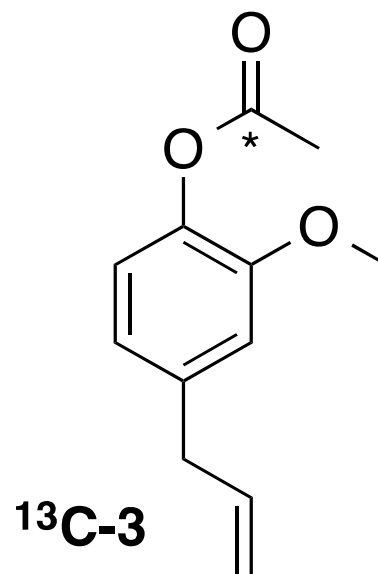
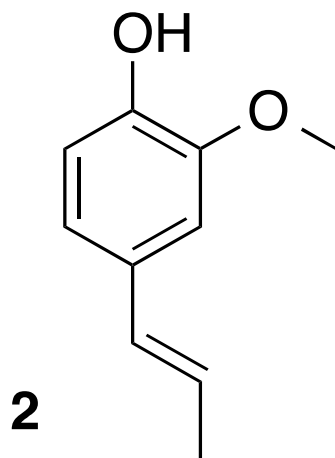
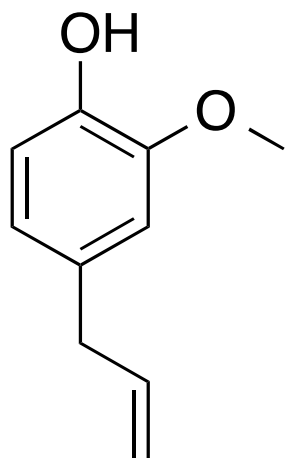
The iso/eugenyl acetates story...

- ❑ The mechanism underlying this observation is still not clear...
- ❑ It can be hypothesized that isoeugenyl esters are hydrolyzed either enzymatically (epidermal esterase) or chemically (hydrolysis)...
- ❑ This hypothesis can be supported by the Castro et al. study showing *in vitro* that skin cytosol/microsomes could hydrolyze isoeugenyl/eugenyl acetate into their parent compounds...



The iso/eugenyl acetates story...

- **Carbon-13 substituted iso/eugenyl acetates were synthesized**
 - **To increase the sensitivity...**
 - **To discriminate between acetates released by iso/eugenyl derivatives and other acetates...**





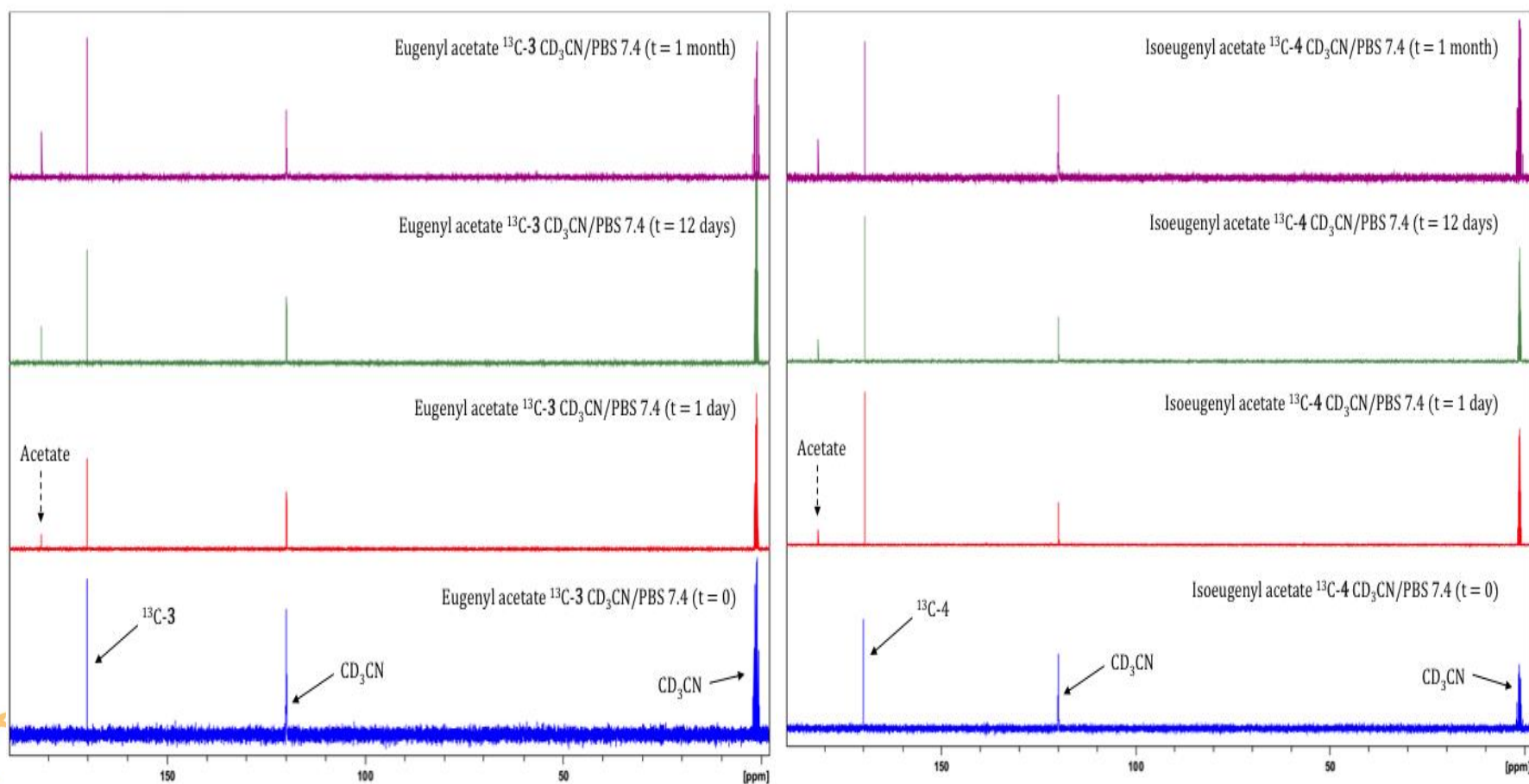
The iso/eugenyl acetates story...

- ❑ **The stability of eugenyl and isoeugenyl acetates was first assessed in a 1:3 mixture of acetonitrile and phosphate buffer (PBS pH 7.4)...**
- ❑ **Reactions were followed by ^{13}C NMR over a period of one month...**
- ❑ **Both eugenyl and isoeugenyl acetates were found to be rather stable toward chemical hydrolysis with only a slow release of free acetate over time...**



The iso/eugenyl acetates story...

- **Stability of iso/eugenyl acetates in a 1:3 mixture of acetonitrile and PBS pH 7.4**





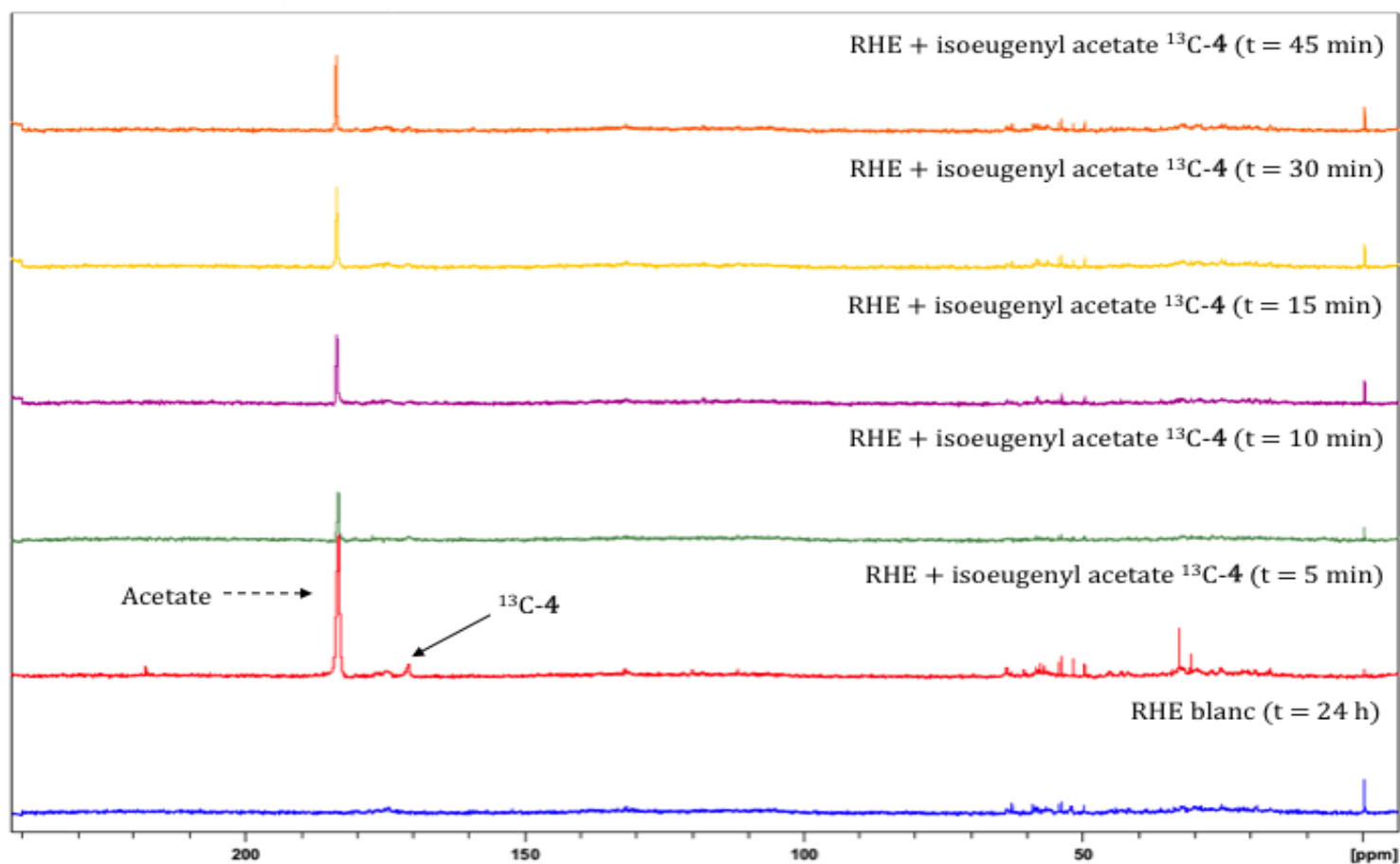
Model 1: iso/eugenyl acetates

- ❑ **A set of experiments was carried out with isoeugenyl acetate ^{13}C -4 following incubation times of 5, 10, 15, 30 and 45 minutes, respectively...**
- ❑ **Spectra obtained indicate a very fast hydrolysis of isoeugenyl acetate ^{13}C -4...**
- ❑ **Even after 5 min, the residual signal of isoeugenyl acetate (δ 170.8 ppm) was very small with a major signal at δ 183.4 ppm corresponding to the hydrolyzed acetate ...**



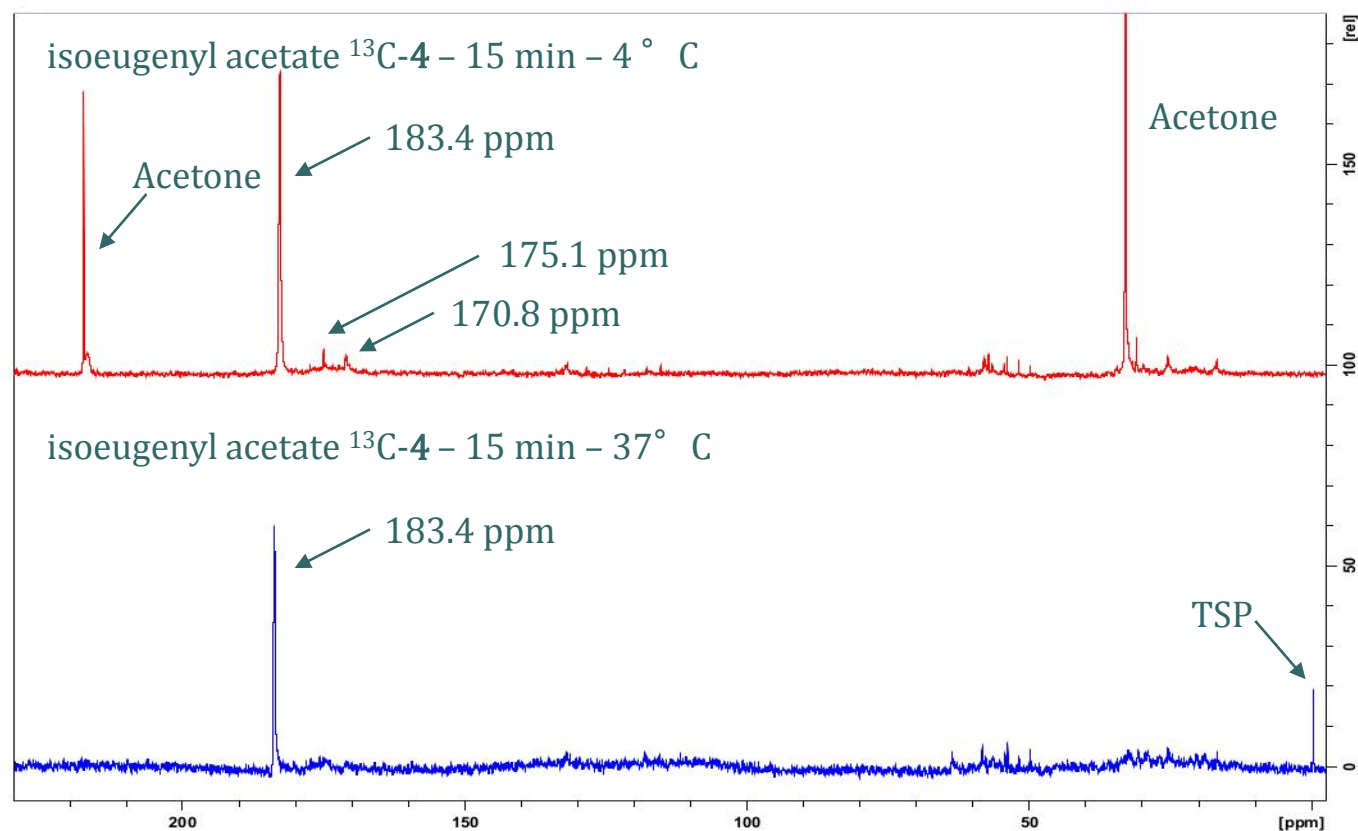
The iso/eugenyl acetates story...

□ Stability of isoeugenyl acetate on RHE



The iso/eugenyl acetates story...

- **Stability of isoeugenyl acetate on RHE at 4° C**





what do we know?
what do we guess?
what do we just ignore?
... and a raccoon?



IDEA Workshop
Pre- and pro-haptens
Leuven, 20th-21st October 2015