Clinical studies, dermatological observations and toxicological considerations on haptens occurring from the biotic transformation of fragrance allergens…

Prof. Jean-Pierre Lepoittevin
University of Strasbourg

IDEA Workshop
Risk Assessment of Pre- & Prohaptens
Dolce la Hulpe Brussels, May 28-29, 2013

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Contact Dermatitis 2006: 54: 73–74
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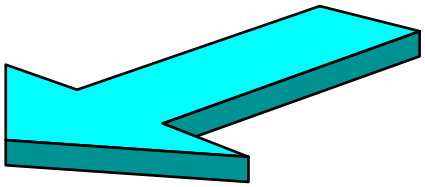
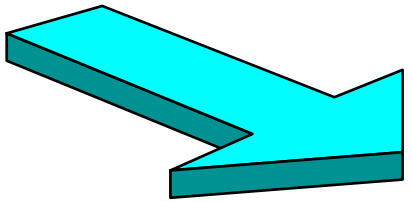
CONTACT DERMATITIS

Editorial

Metabolism *versus* chemical transformation or pro- *versus* prehaptens?



Alternative methods...



Activation mechanisms...

Lepoittevin, J.-P. Contact Dermatitis, 2006, 54, 73-74

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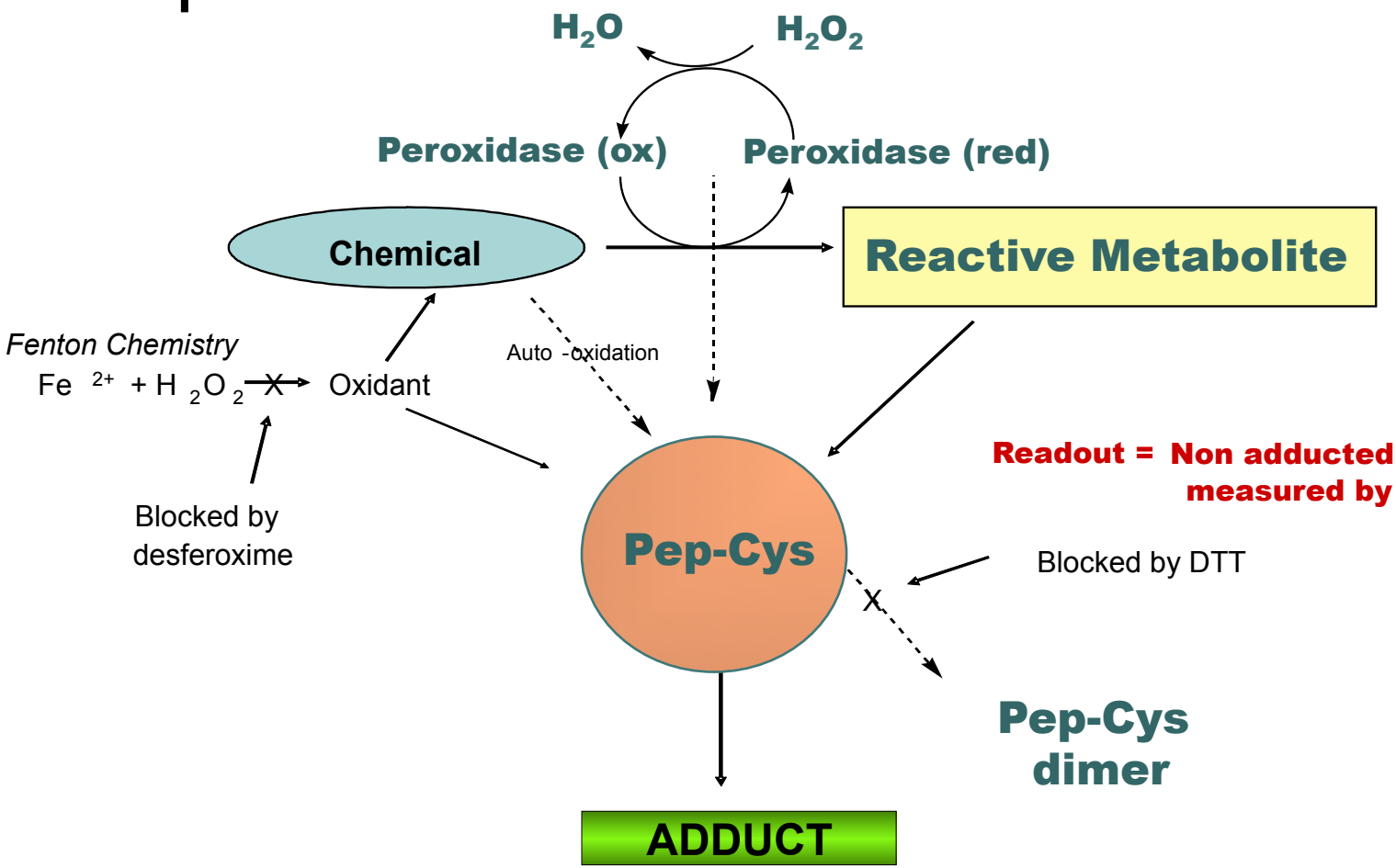
Peroxidase Peptide Reactivity Assay…



Readout = Non adducted peptide monomer measured by MS

Monitored in MS Assay but not quantified vs. monomer

Gerberick GF et al. Toxicol Sci 2009, 112, 164-174

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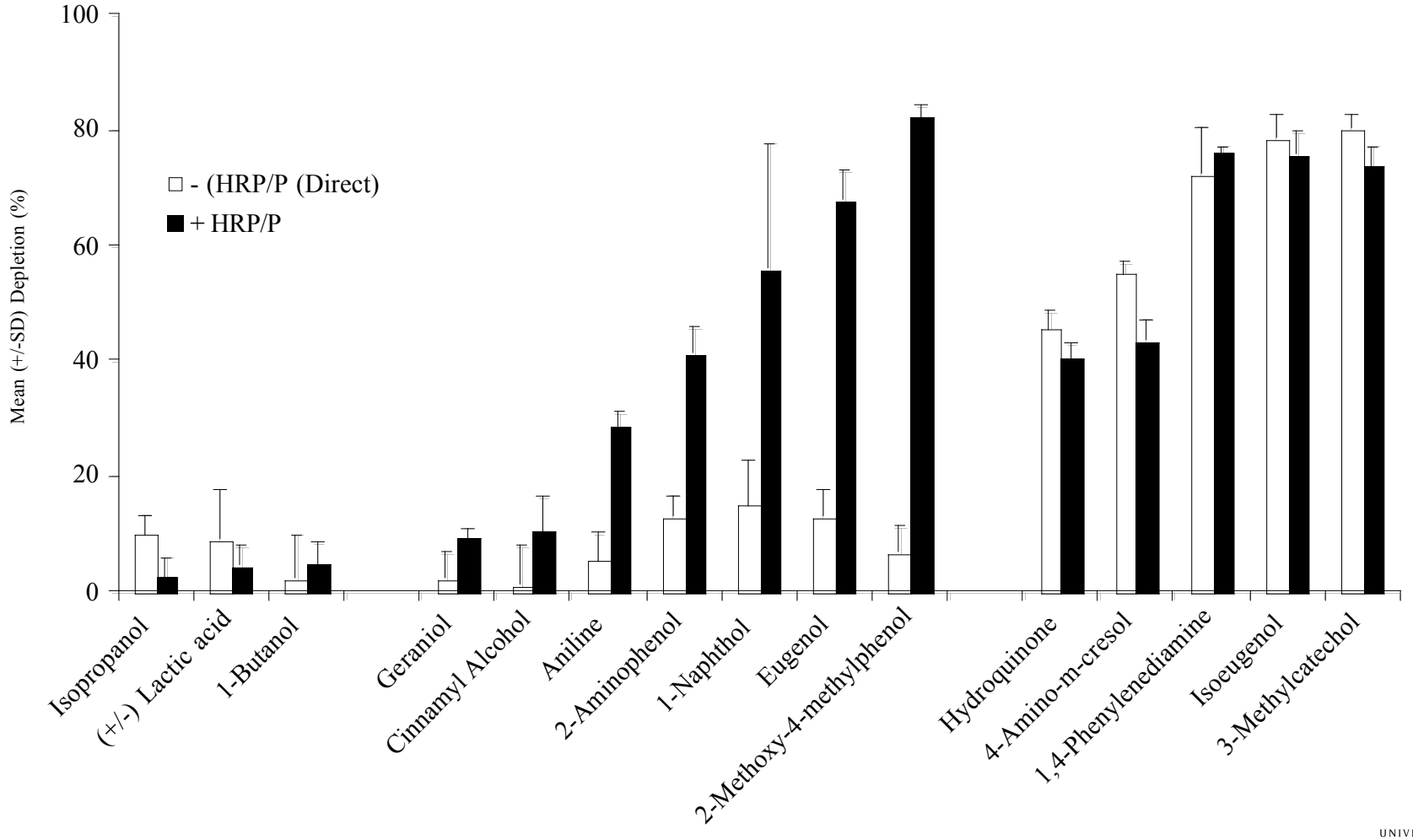
Peroxidase Peptide Reactivity Assay…



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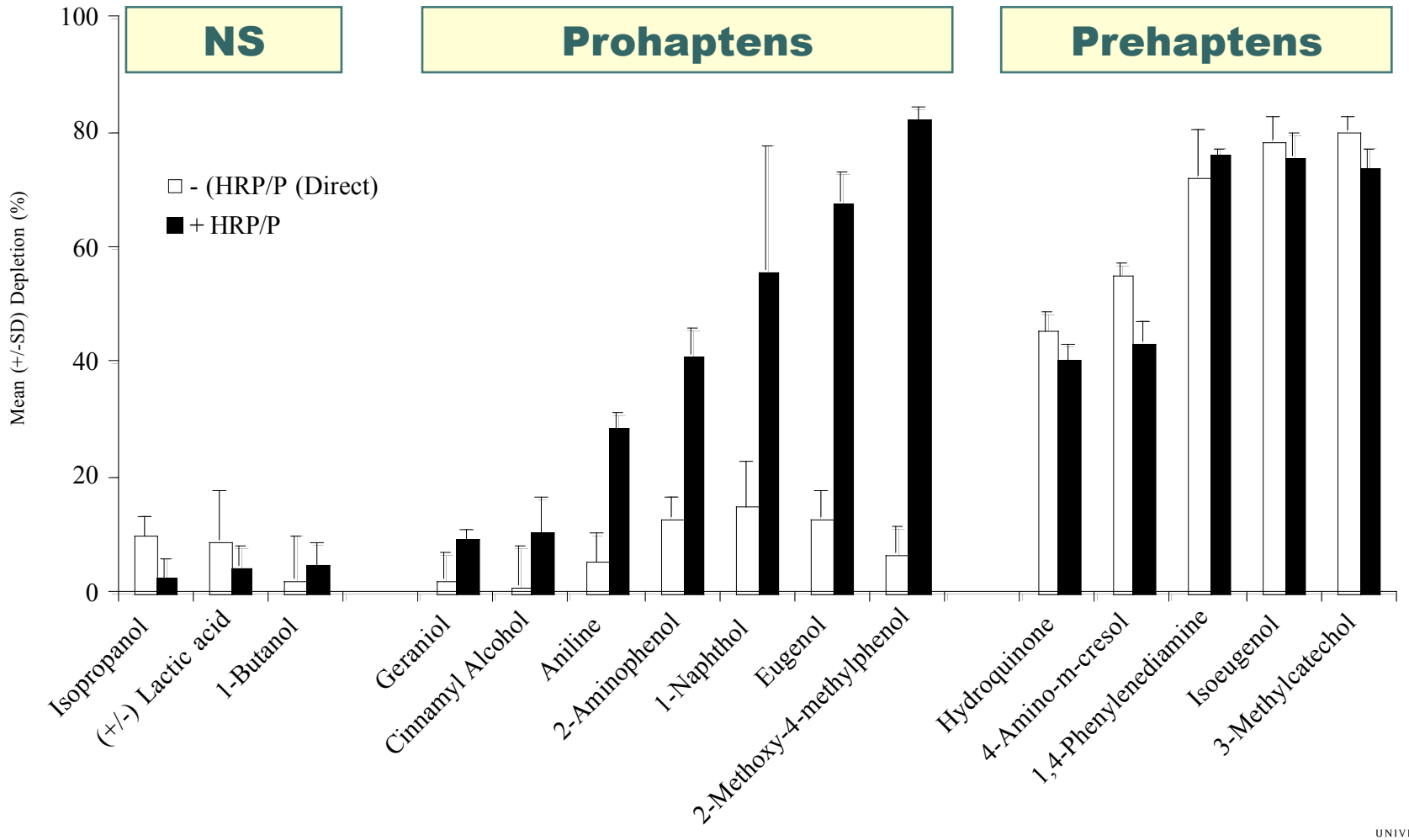
Peroxidase Peptide Reactivity Assay…



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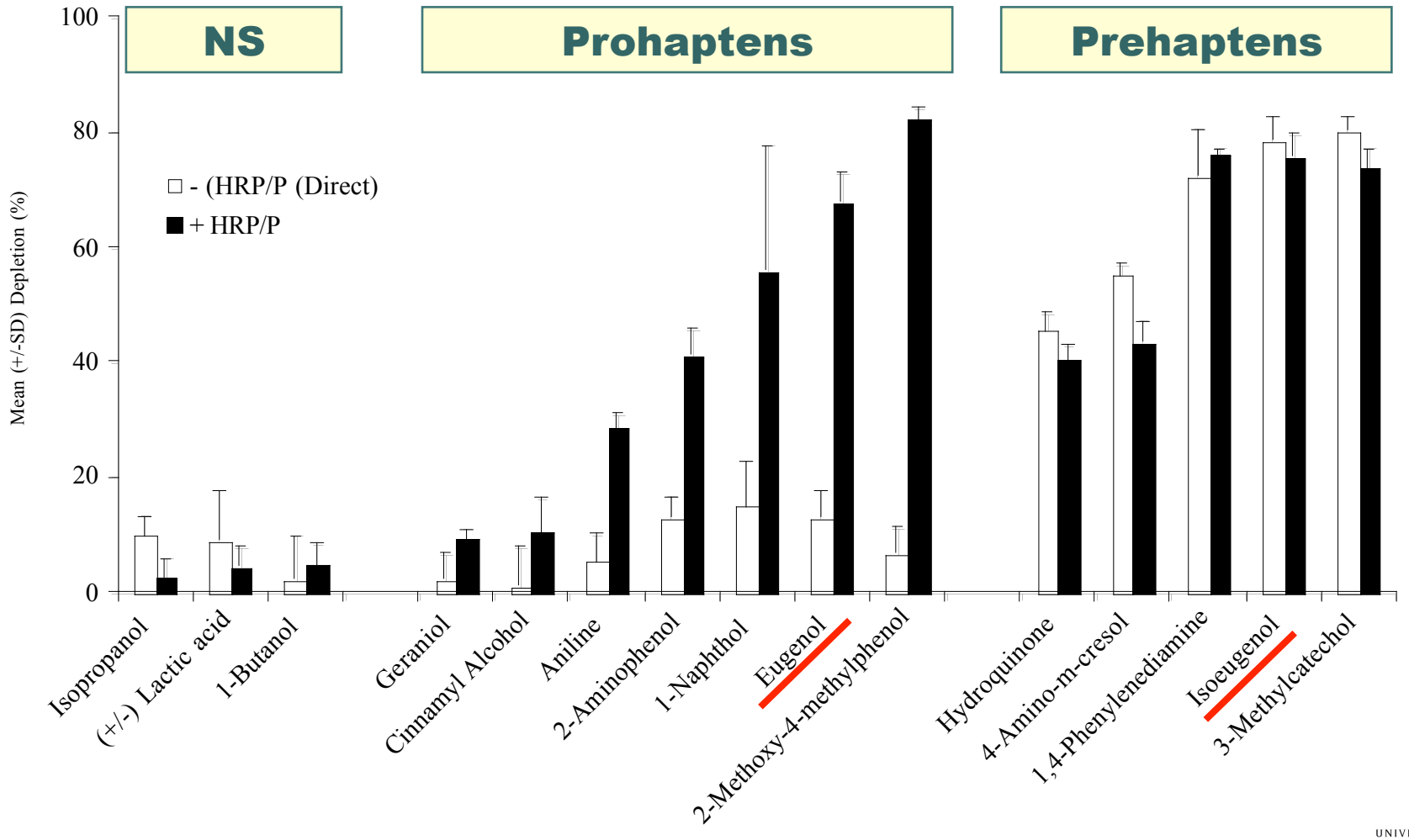
Peroxidase Peptide Reactivity Assay…



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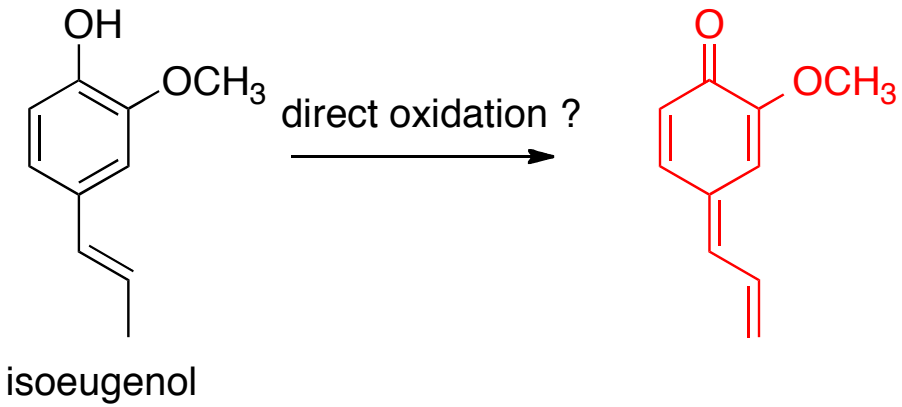
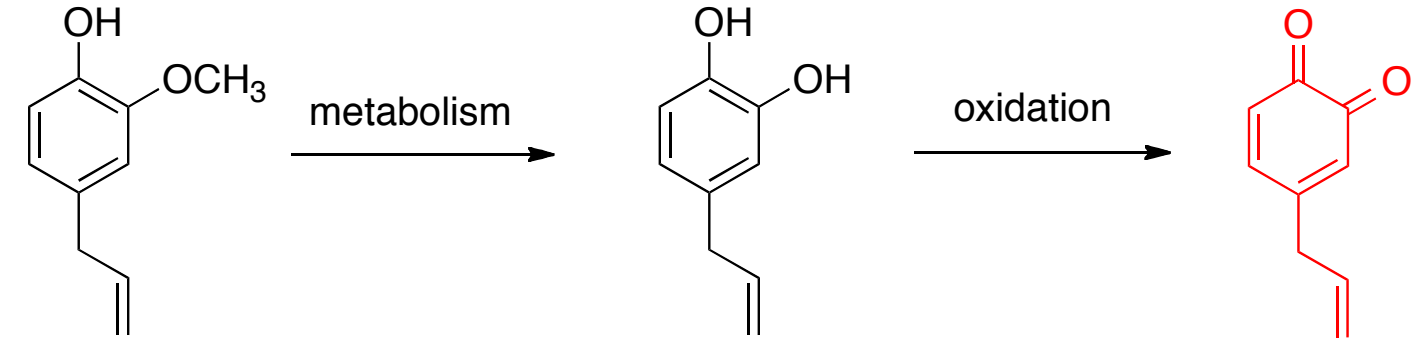
Pro- versus prehaptens...



isoeugenol



eugenol

Bertrand et al. Chem Res Toxicol 1997, 10, 335-343

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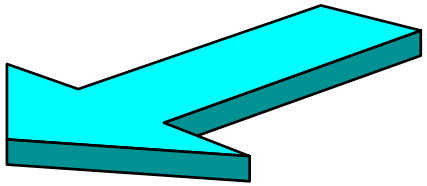
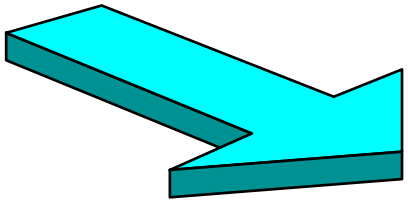
Prohaptens ?



Modified by skin metabolism…



Reactive metabolites…

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Metabolism of xenobiotics

Organe	Cellular type
Liver	Hepatocytes
Kidney	Tubular cells
Lung	Clara cells
Intestine	Mucosa lining cells
Skin	Epidermal cells
Testes	Seminiferous tubules

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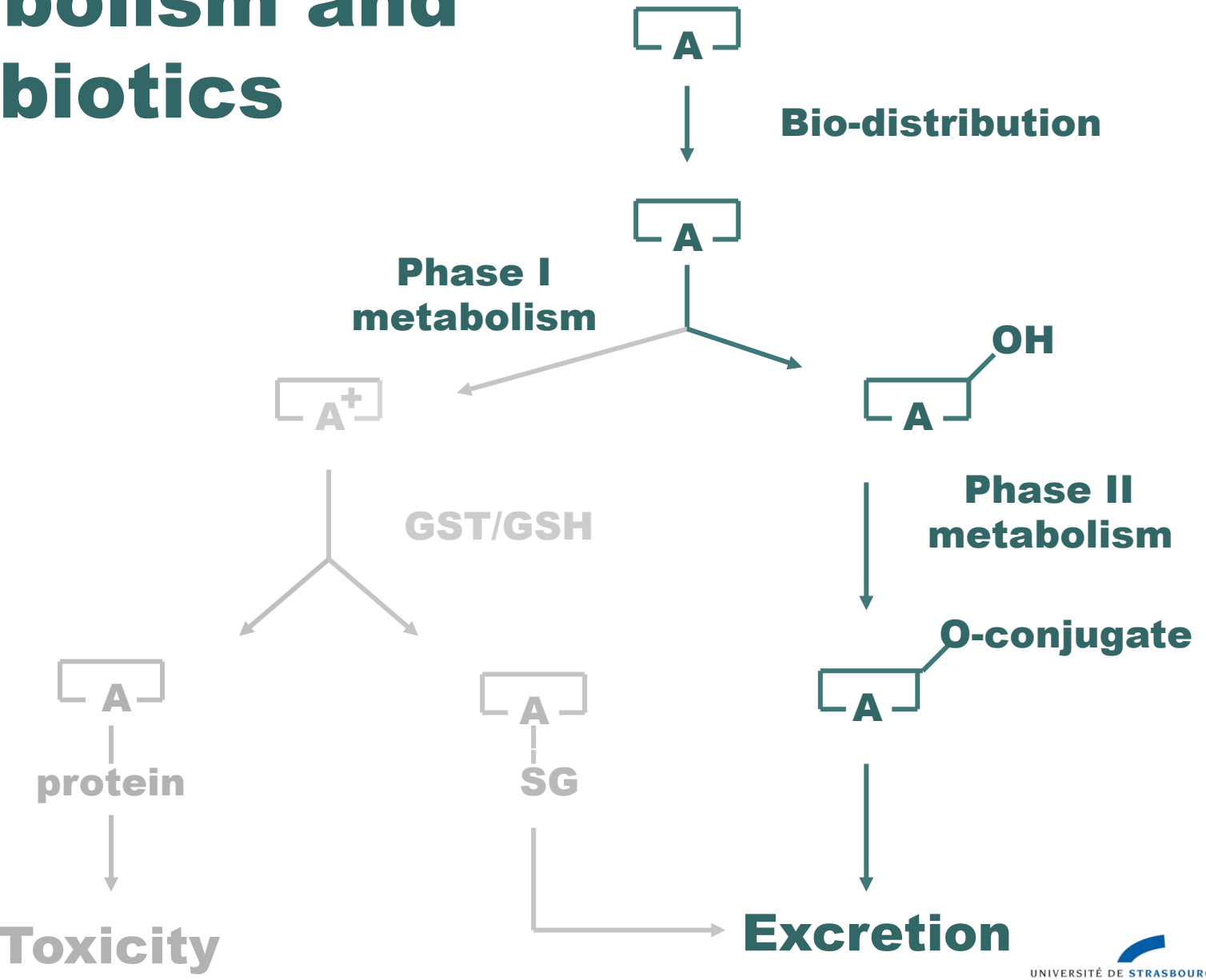
Metabolism and xenobiotics



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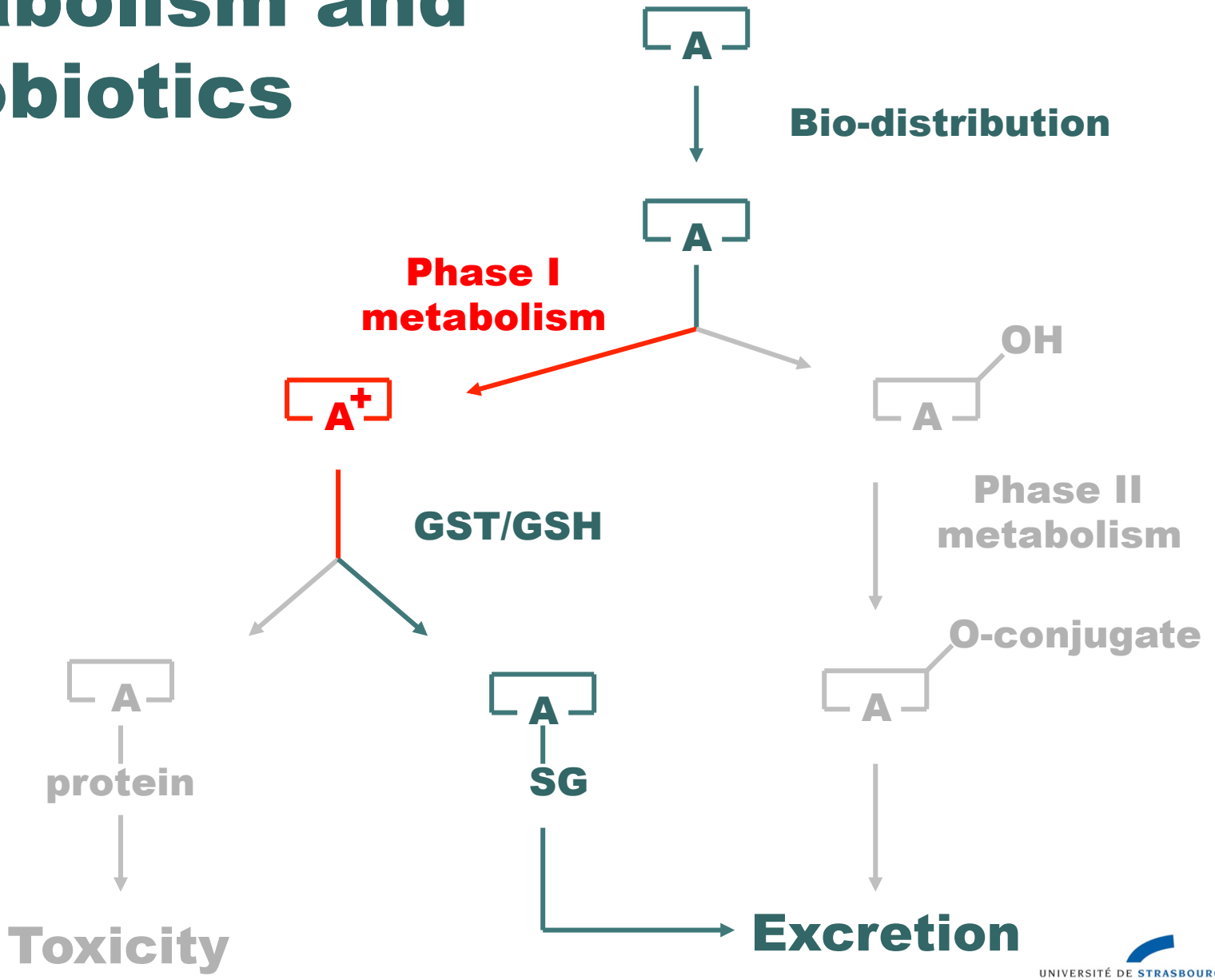
Metabolism and xenobiotics



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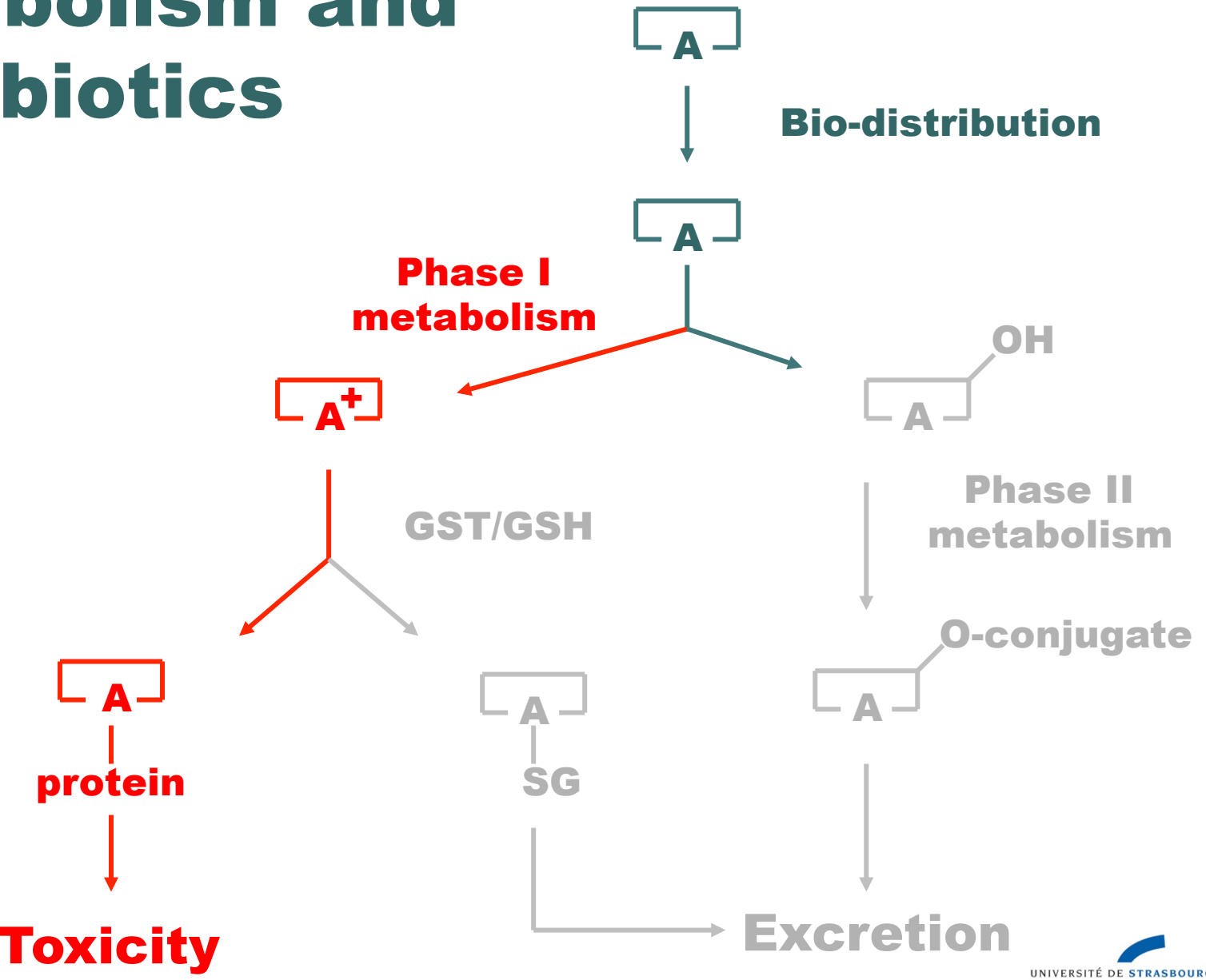
Metabolism and xenobiotics



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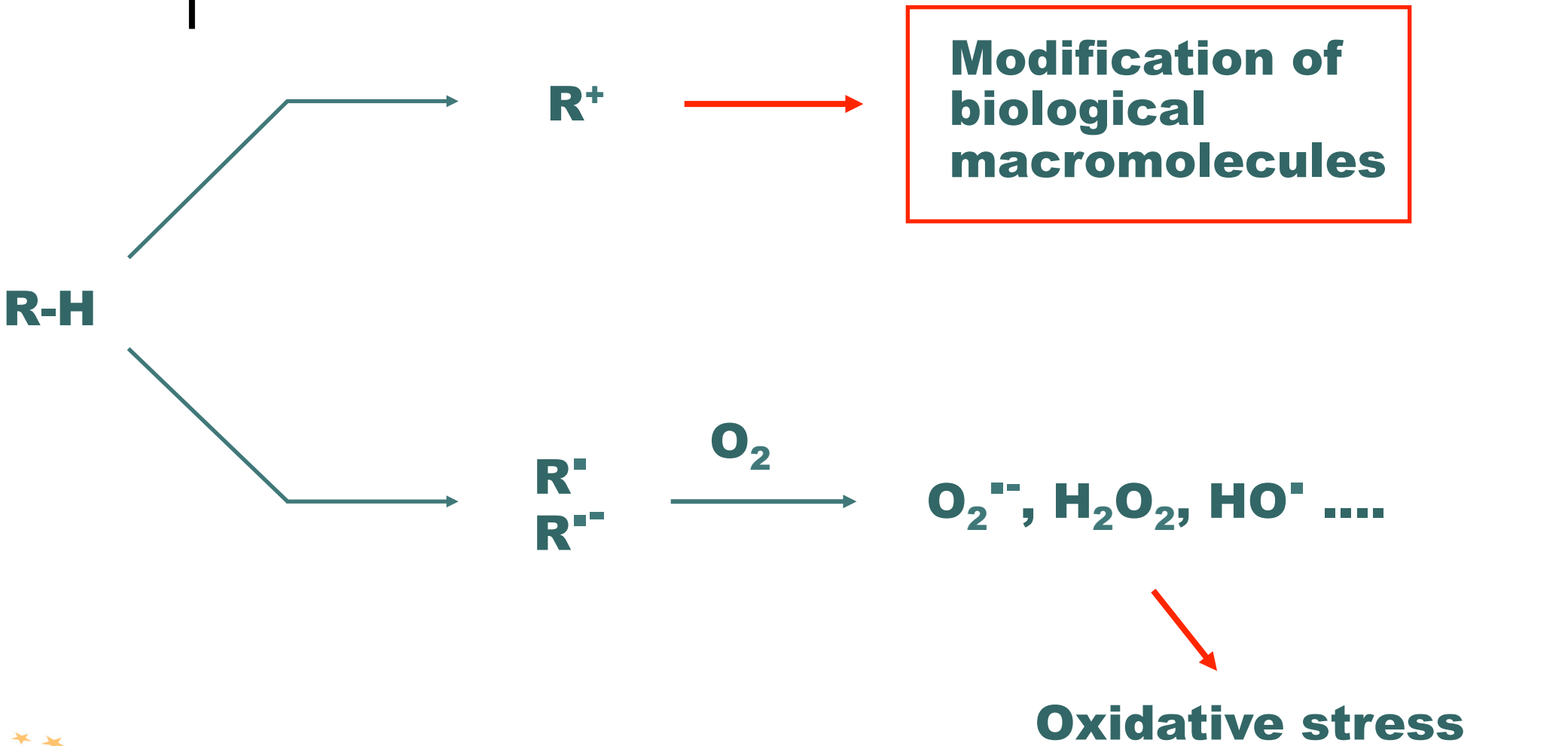
Metabolism of xenobiotics



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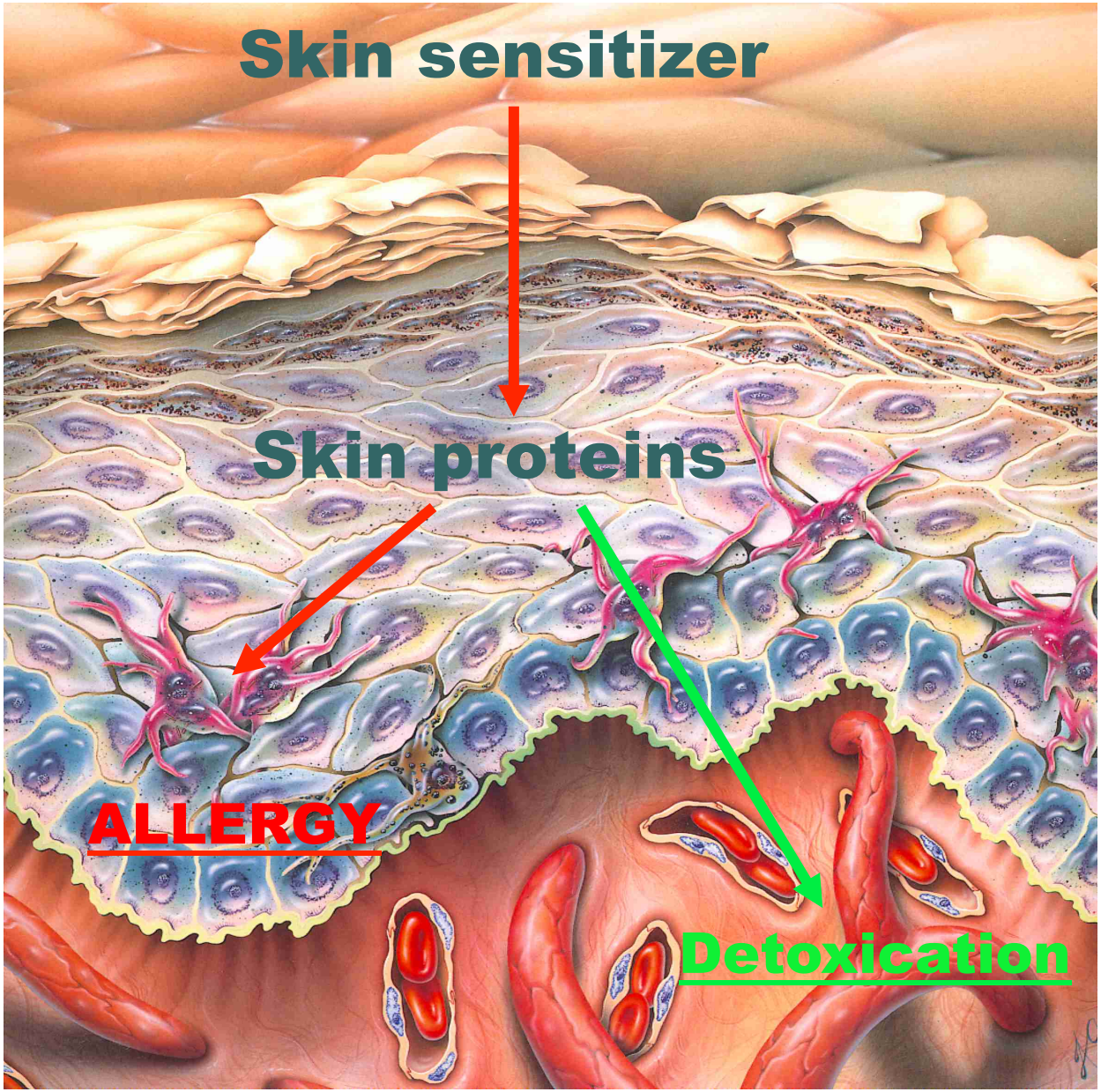
Model



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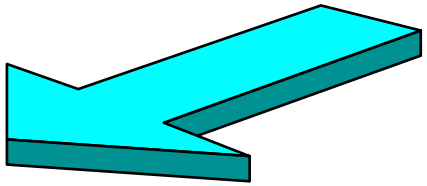
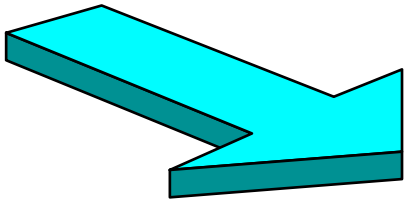
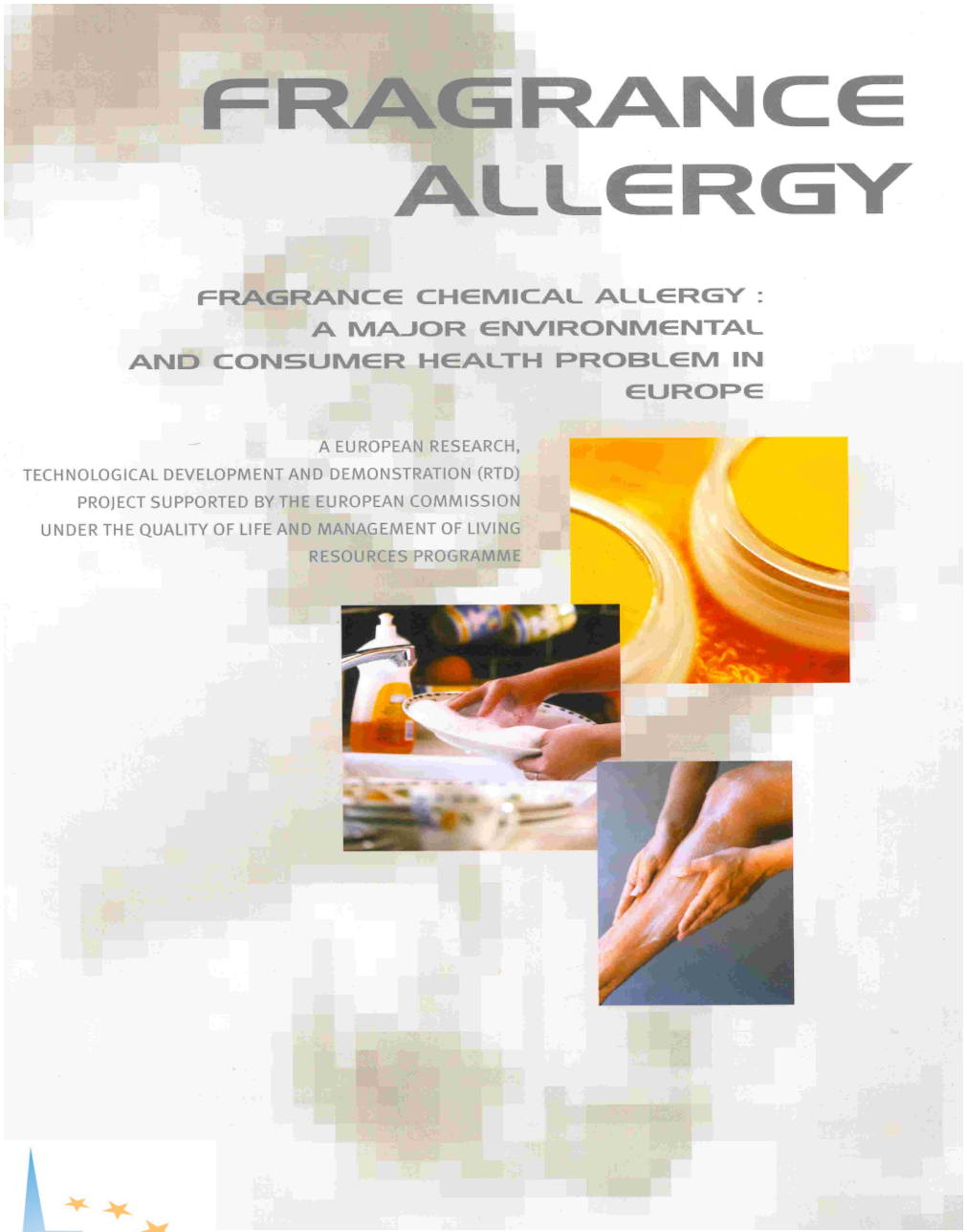
Clinical studies



Cross-reactions…



Co-sensitization…



Participants

EU QLK4-CT-1999-01558

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* University of Witten/Herdecke, D
* St Thomas Hospital, London, UK
* NERI, Roskilde, DK
* University of Odense, DK
* University of Malmö, S
* University of Leuven, B

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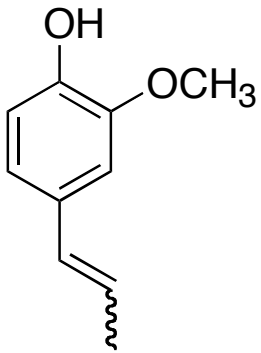
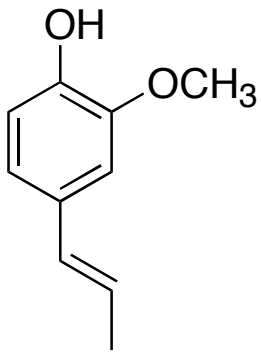
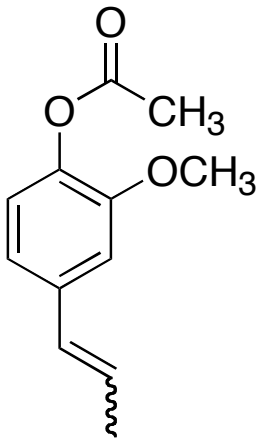
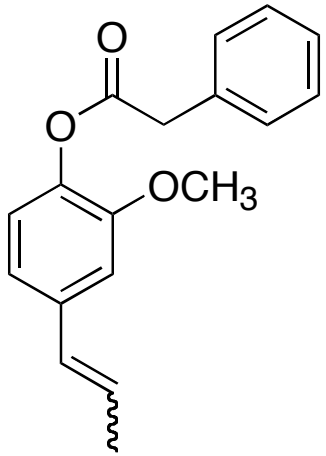
Fragrance substitution…

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

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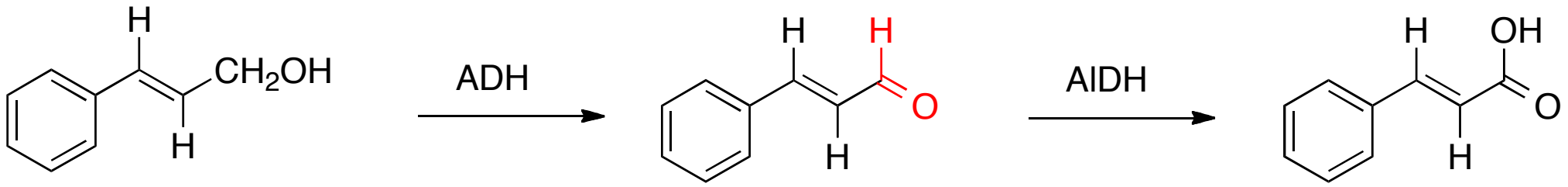
The cinnamic alcohol story…



cinnamic alcohol — cinnamic aldehyde — cinnamic acid

- **“The model” of prohapten for many years…**
- **Pathway demonstrated by many metabolic studies…**

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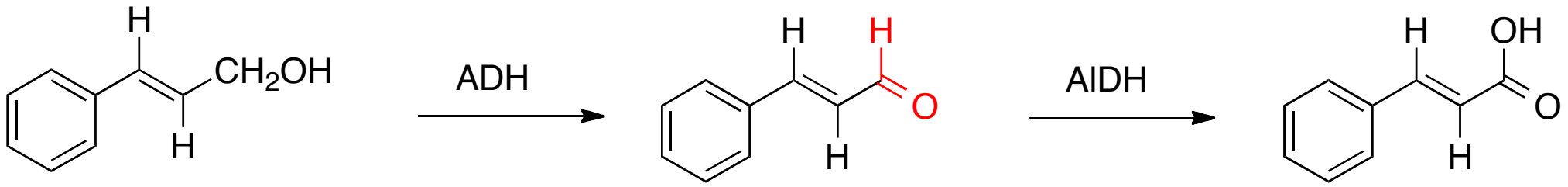
The cinnamic alcohol story…



cinnamic alcohol — cinnamic aldehyde — cinnamic acid

However not all patients sensitive to cinnamic alcohol are reacting to cinnamic aldehyde…

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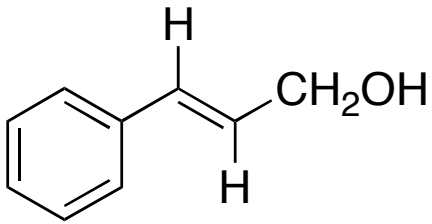
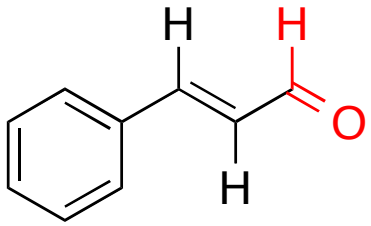
The cinnamic alcohol story…



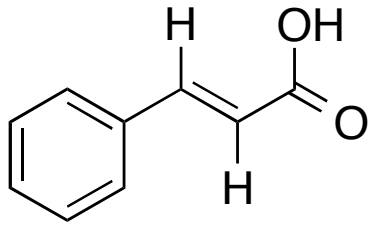
cinnamic alcohol

ADH



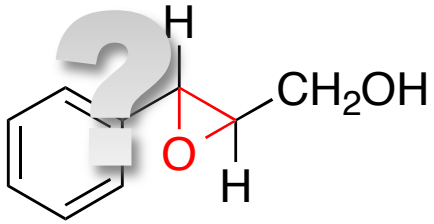
cinnamic aldehyde

AlDH



cinnamic acid

?



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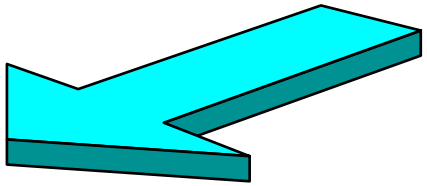
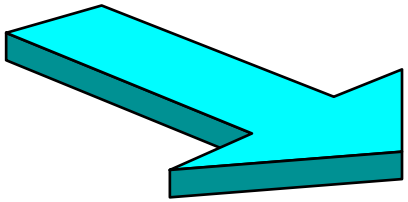
Metabolic studies

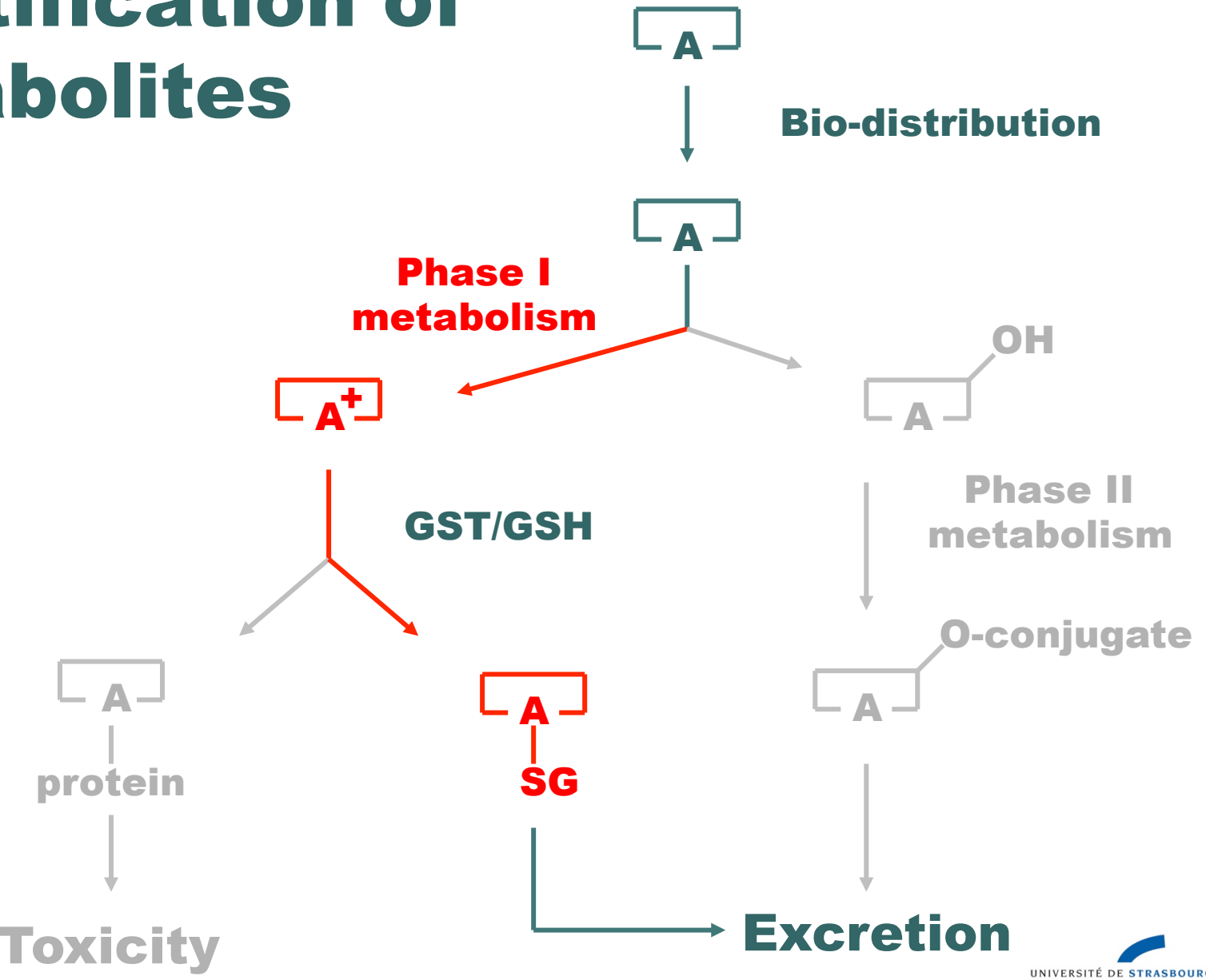


Liver metabolism…

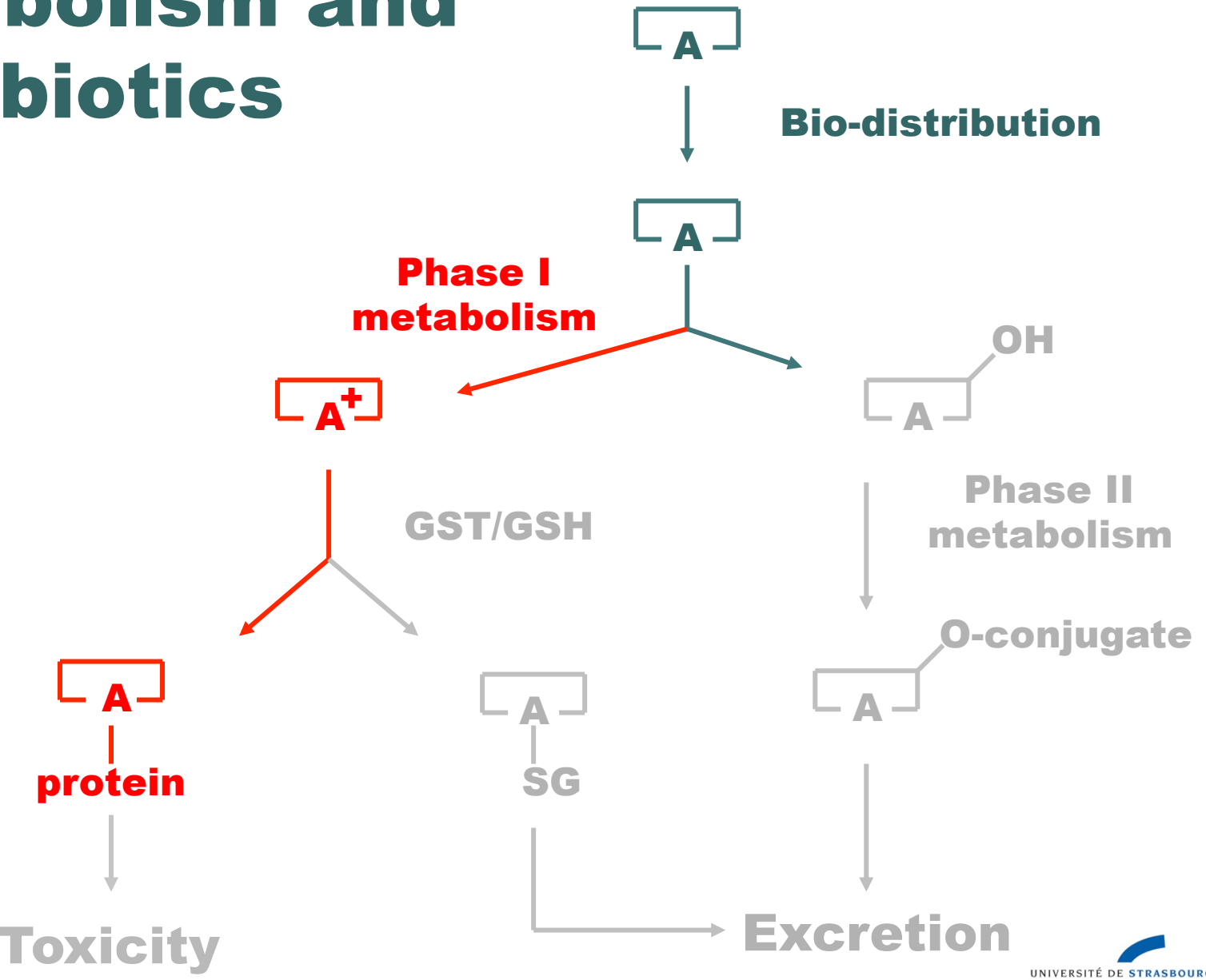


Skin metabolism…

Identification of metabolites



Metabolism and xenobiotics



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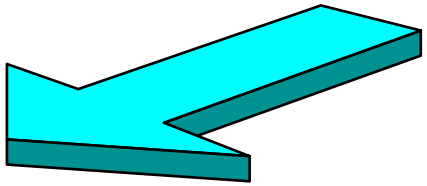
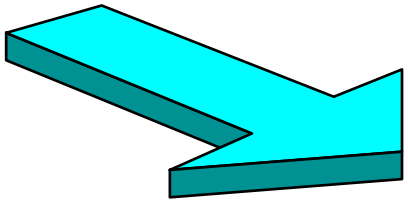
Two major pitfalls



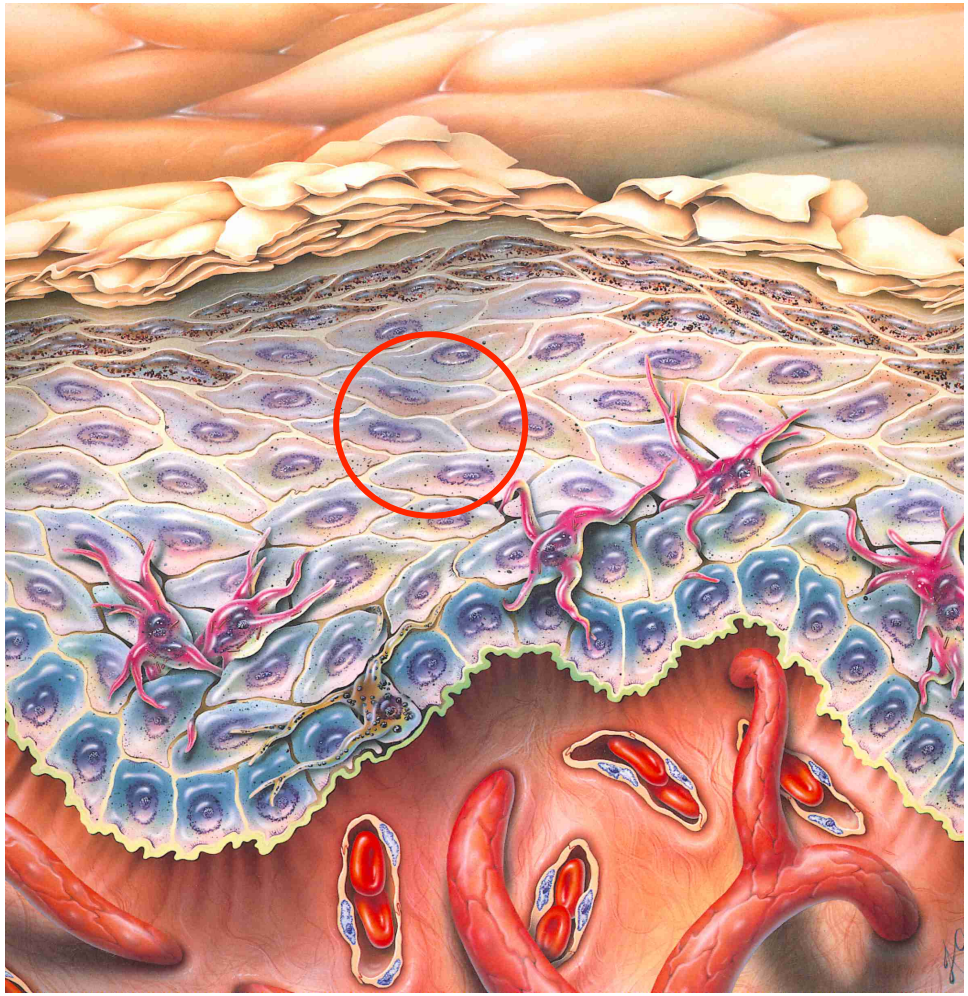
Liver vs skin…



Highly reactive intermediates…

Non invasive approach…

- Direct observation…
- Highly non homogeneous environment…



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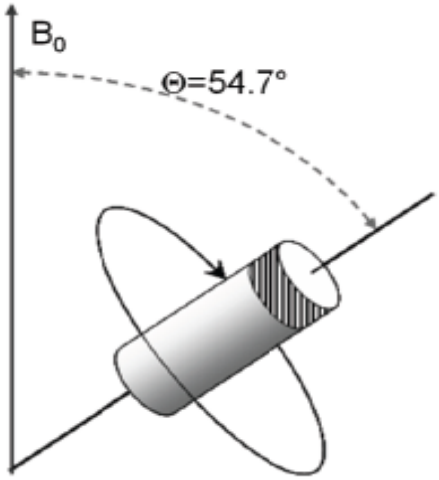
HR-MAS NMR…

- High-Resolution Magic Angle Spinning NMR…
- Bring to zero inhomogeneity associated with the sample…



HR-MAS NMR…

- NMR technique adapted to semi-solid structures…
- Developed for biopsies of soft tissues (mainly tumors)…
  - Brain (1997)
  - Liver, Kidneys (2000)
  - Prostate (2004)
  - Muscles (2005)
  - Breast (2007)…

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HR-MAS NMR…

- Well adapted for the observation of metabolites…
- Classical NMR techniques can be adapted…
  - 1D ^{1}H (10 min)
  - 2D ^{1}H-^{1}H homonuclear (30 min)
  - 2D ^{1}H-^{13}C heteronuclear (several hours)
  - Other nuclei are observable (^{31}P, ^{15}N, ^{19}F…)

Is it possible to follow a reaction between a xenobiotic molecule and Reconstructed Human Epidermis ?

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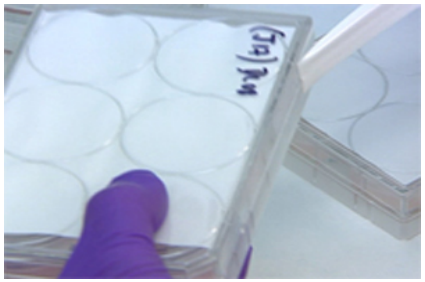
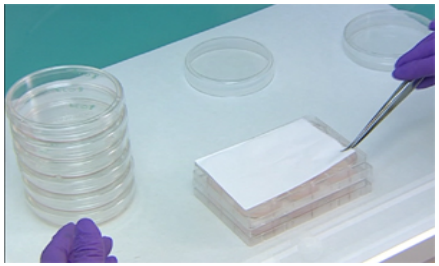
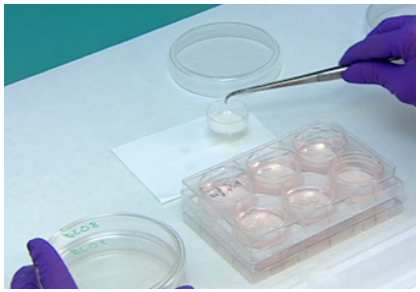
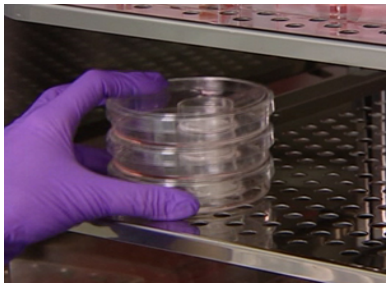
HR-MAS NMR on RHE (SkinEthic®)

- RHE are incubated for 2 h at 37°C, CO_2 5%...

- Hapten in acetone (100 µL) is applied on skin surface (4 cm^2) and RHE are then incubated for various periods (2h, 4h, ..., 24h, 48h, etc.)...

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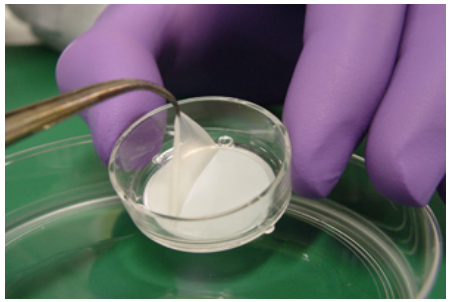
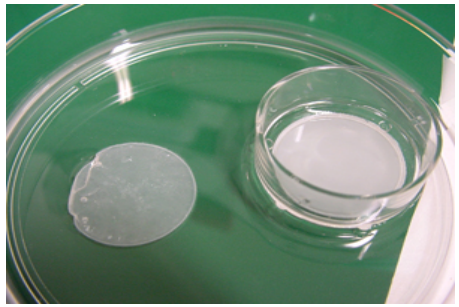
HR-MAS NMR on RHE (SkinEthic®)

- RHE are treated with Dispase II to remove the matrix…





- RHE are washed, frozen at -80°C and sampled for HR-MAS NMR experiments…

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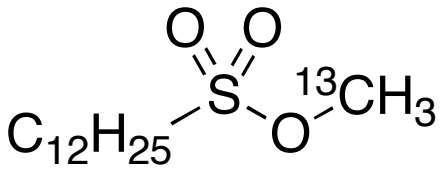
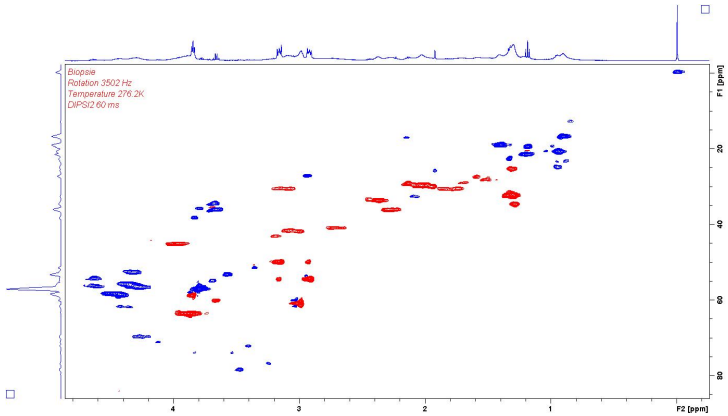
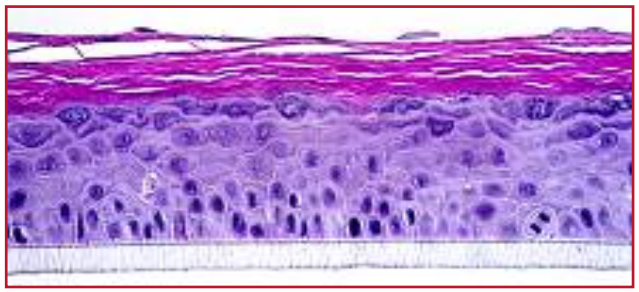
HR-MAS NMR on RHE (SkinEthic®) (^{13}C)-methyl-C12-sulfonate





0.06 mmol / 100 µL acetone, 4 cm²
Incubation 24h or 48h



Elbayed K., et al. Chem Res Toxicol 2013, 26, 136-145

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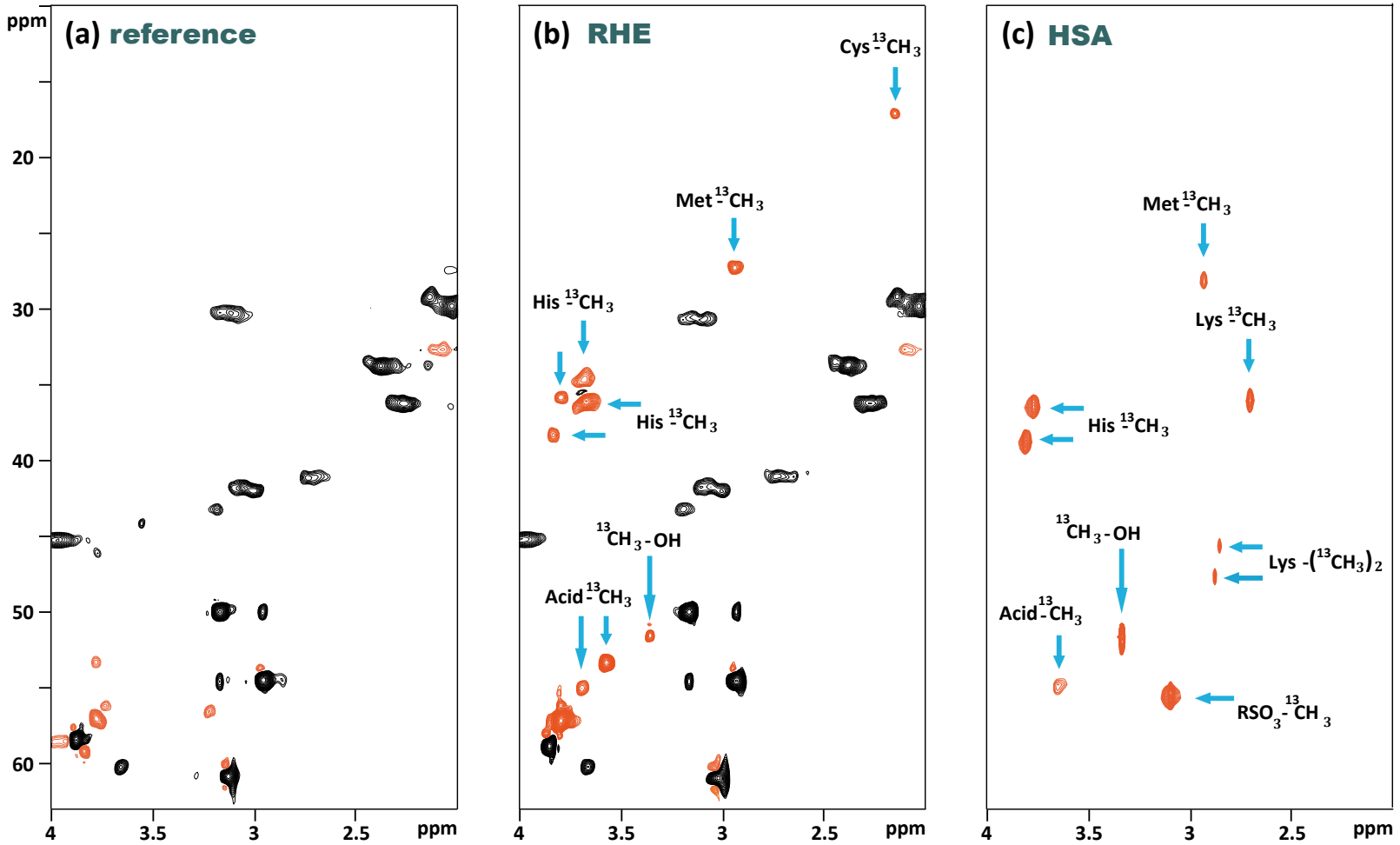
HR-MAS NMR on RHE (SkinEthic®) (^{13}C)methyl C12-sulfonate…



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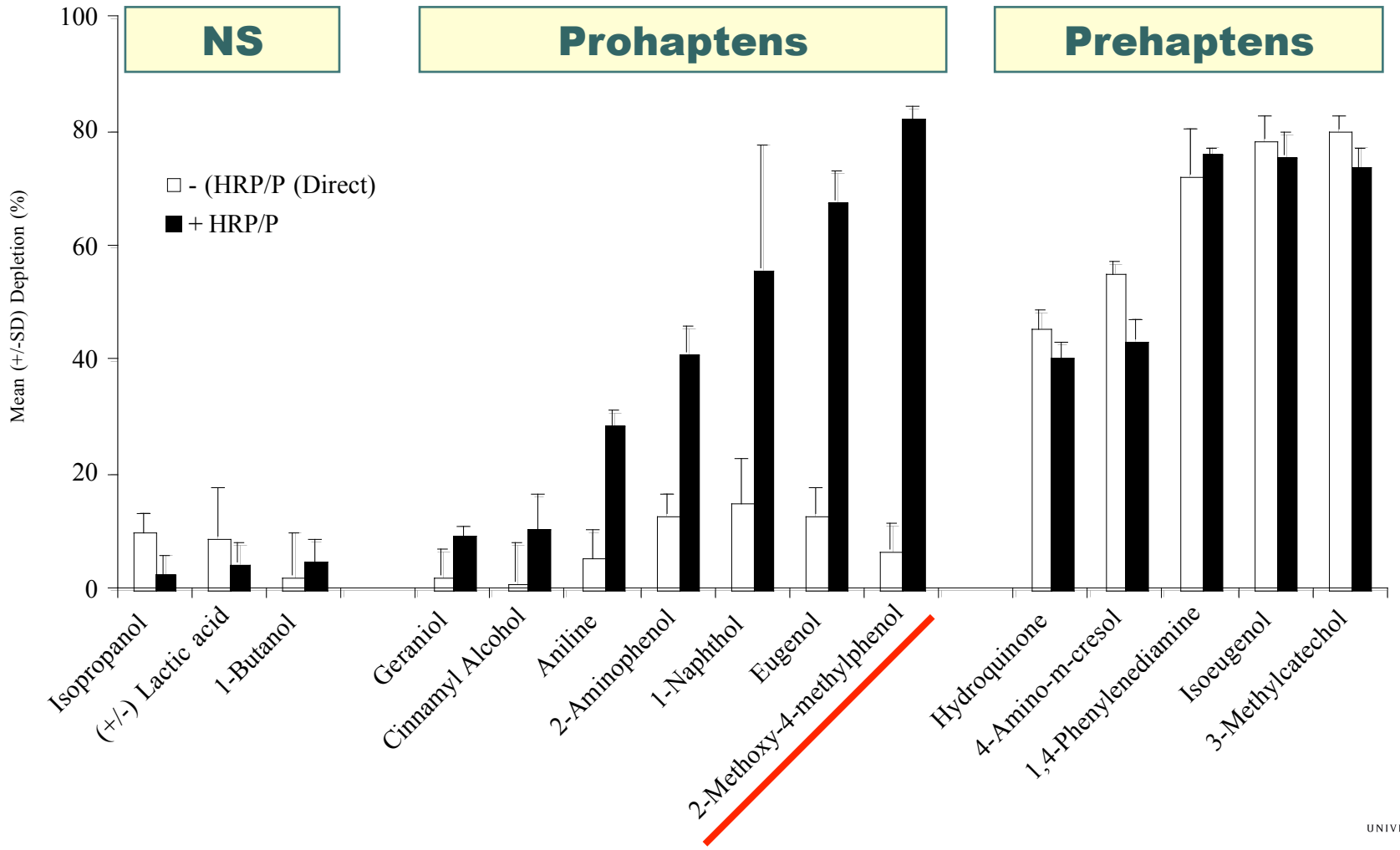
Peroxidase Peptide Reactivity Assay…



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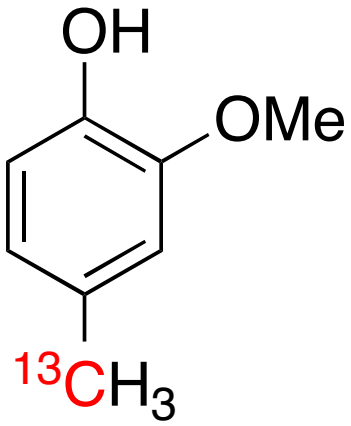
Model of prohaptens...



- 43rd Amendment IFRA
- weak sensitizer
- WoE NESIL 118 μg/cm²

2-methoxy-4-methylphenol
2M4MP

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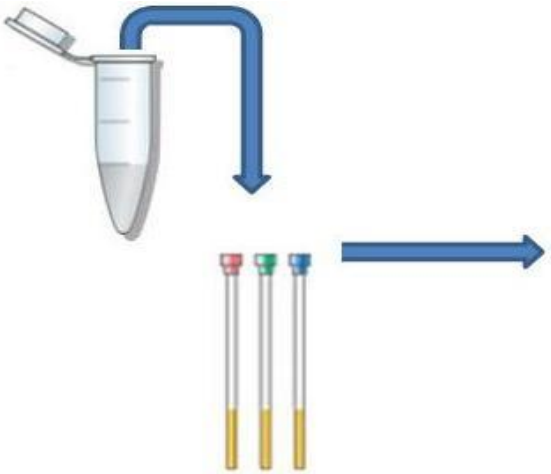
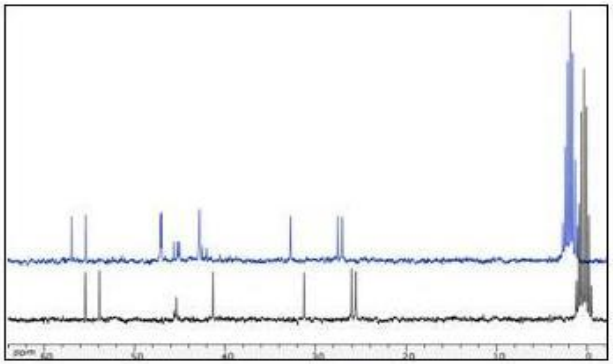
H_2O_2 / HRP Peptide Reactivity…

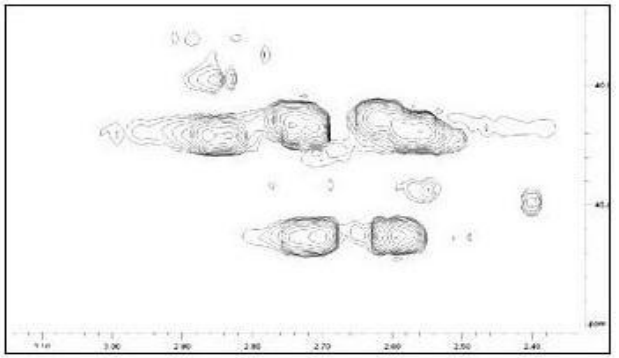
Chemical = 1 mg / Solvent = 600 µL
H_2O_2 = 1.5 equiv. / HRP = 65.7 U
Desferroxamine = 0.1 equiv.
Solvent: CH_3CN/H_2O or buffer 1:2
GSH 10 equiv.; H_2O
Pep-Cys 2 equiv.; PBS 7.4
Pep-Lys 2 equiv.; PBS 7.4 and AB 10.2



Reaction medium put into NMR tubes



Reactions followed by 1D ^{13}C NMR



Structures assigned by 2D ^{1}H - ^{13}C HSQC and HMBC NMR

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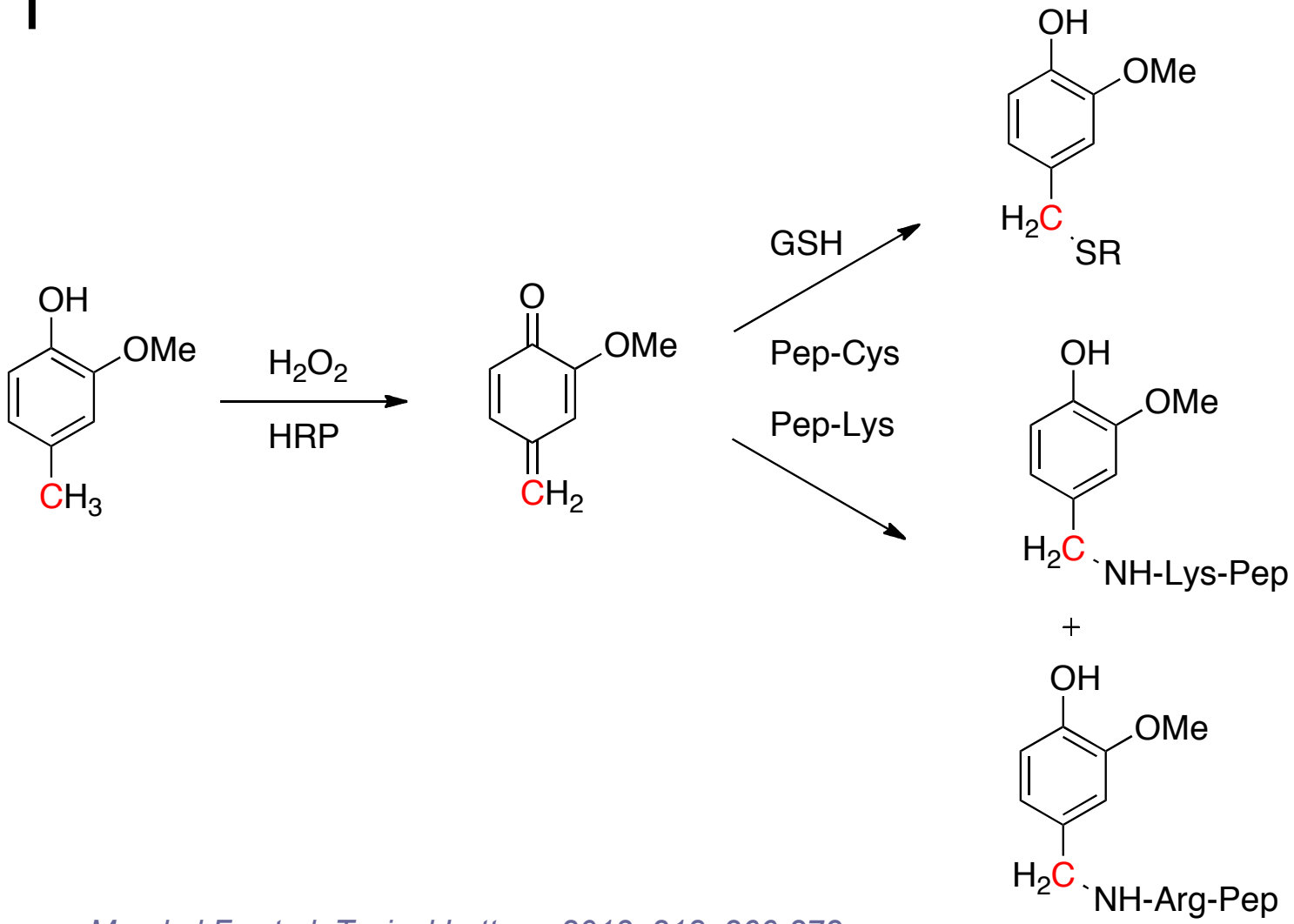
2M4MP + H_2O_2 + HRP + Peptides



Merckel F., et al. Toxicol Letters, 2013, 218, 266-272

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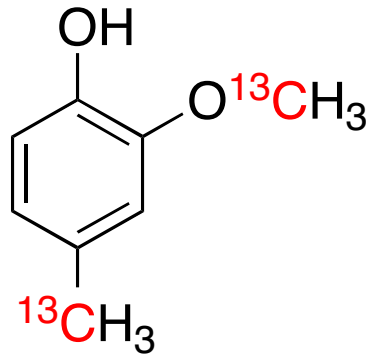
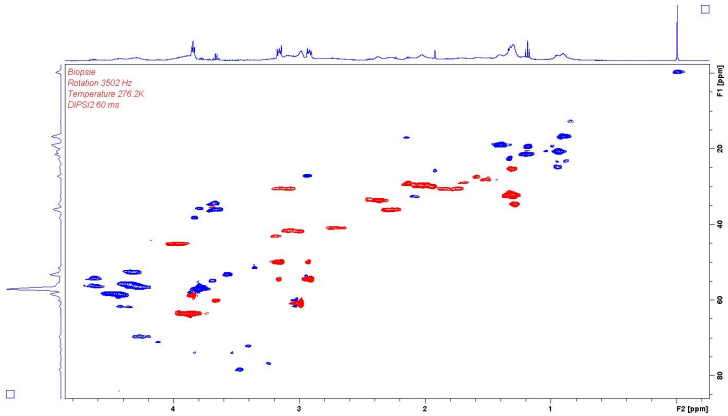
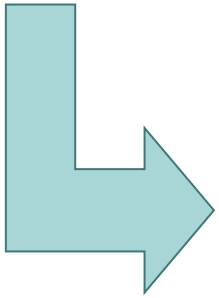
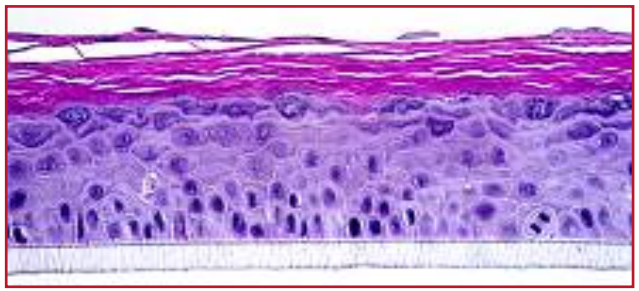
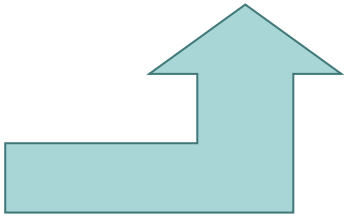
HR-MAS NMR on RHE (SkinEthic®)











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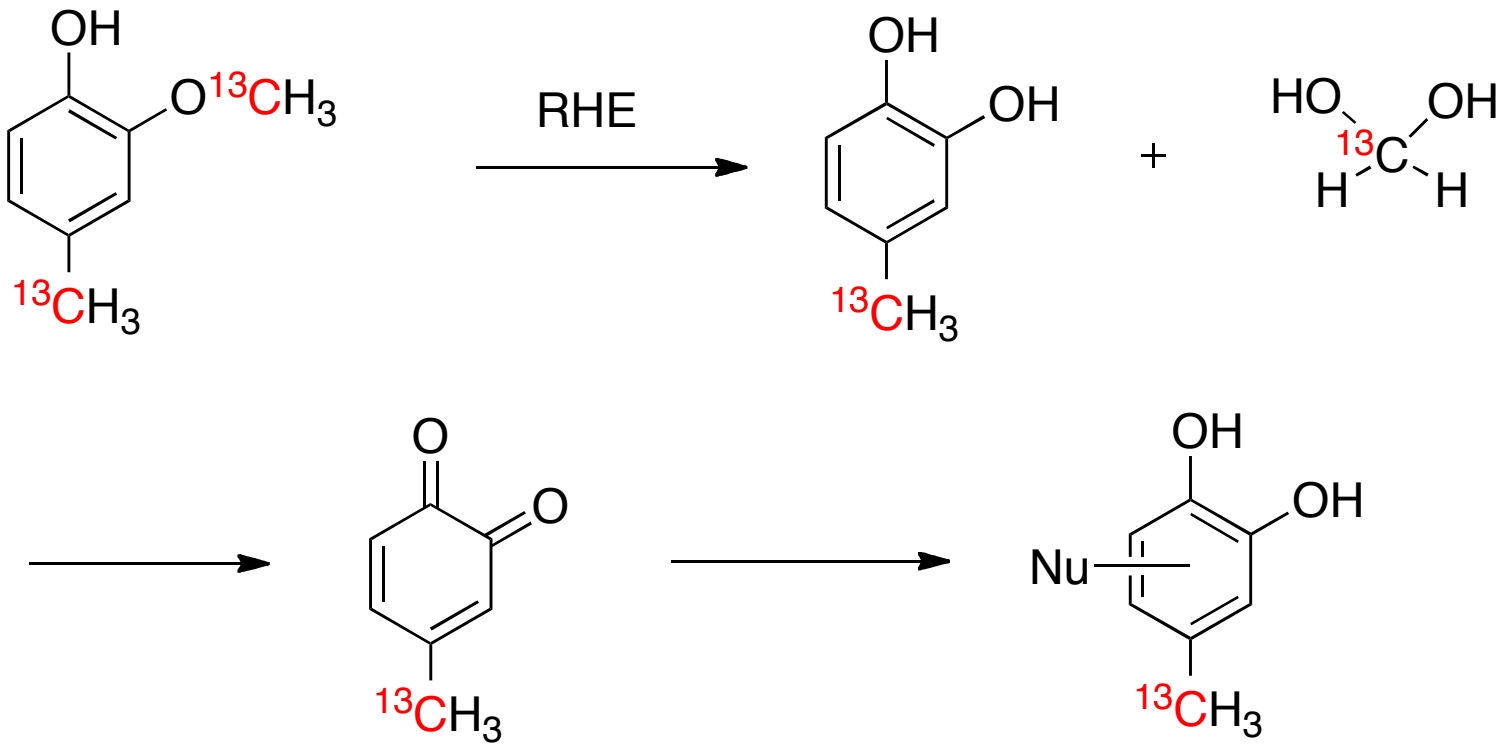
2M4MP + HR-MAS NMR on RHE



Moss E., et al. unpublished results

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Hapten-protein interactions…

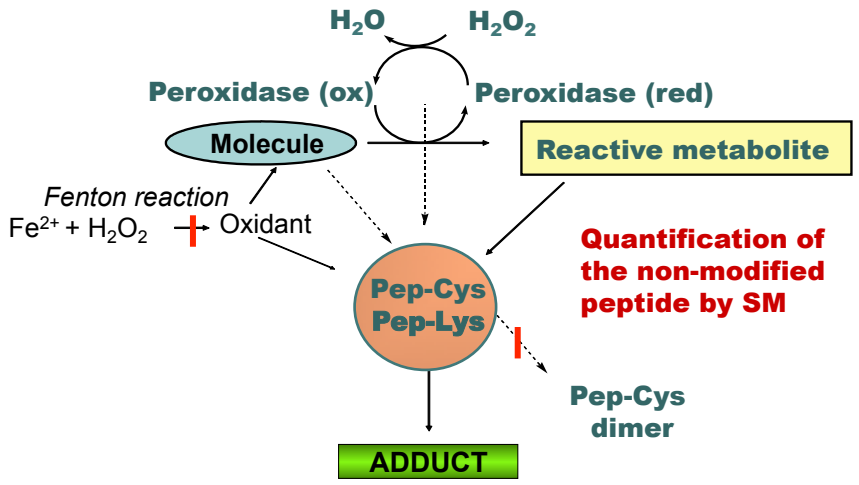
1936 — 1980 — 1990 — 2010

Model nucleophiles: Aniline, thiophenol etc…

Amino-acids, peptides

Model proteins

RHE



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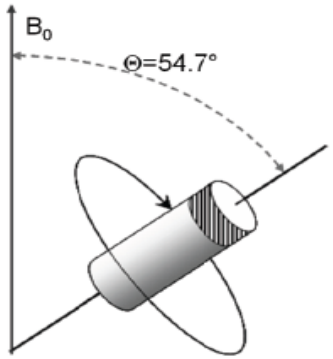
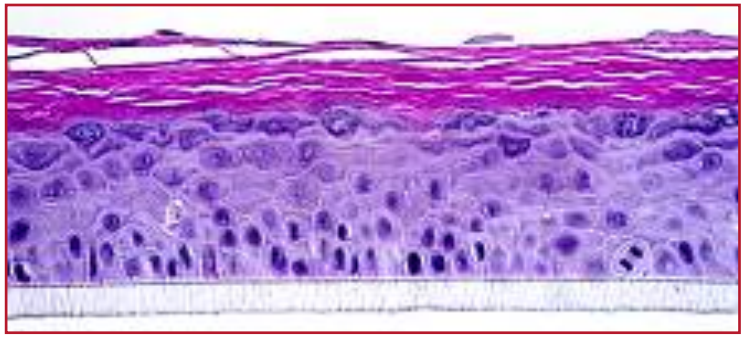


HR-MAS



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Conclusions

- Most chemicals are detoxified through metabolism…
- Reactive intermediates can modify proteins and induce skin allergies…
- Skin metabolism associated with hapten formation is difficult to investigate…
- Mechanisms associated are mainly unknown…

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- Université de Strasbourg
- Ministère de l'Éducation et de la Recherche

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