

# Evaluation of the risk reduction associated with smoking cigarettes producing less free radicals.

Iman EMAMI,

*Biosyntec, Paris, France*

Kroum ALEXANDROV,

*CNRS*

Margarita Rojas,

*CNRS*

Christian ROLANDO,

*Université des Sciences et Technologies de Lille,*



# PLAN

- 1) Why removing free-radicals in cigarette smoke?
- 2) Principle of the new Biosyntec active filter based on *Rosemary* extracts
- 3) Identification and quantification of the free radical decrease
- 4) Ascertaining the effect of smoke free radicals:
  - In vitro* cell culture, DNA level, evaluation of adducts
  - In vivo* Human plasma (oxidized LDL, oxidized proteins)
- 5) Evaluation of the risk reduction associated with smoking cigarettes producing less free radicals.
- 6) Conclusion: innovative products and research are necessary to reduced the smoke toxicity.

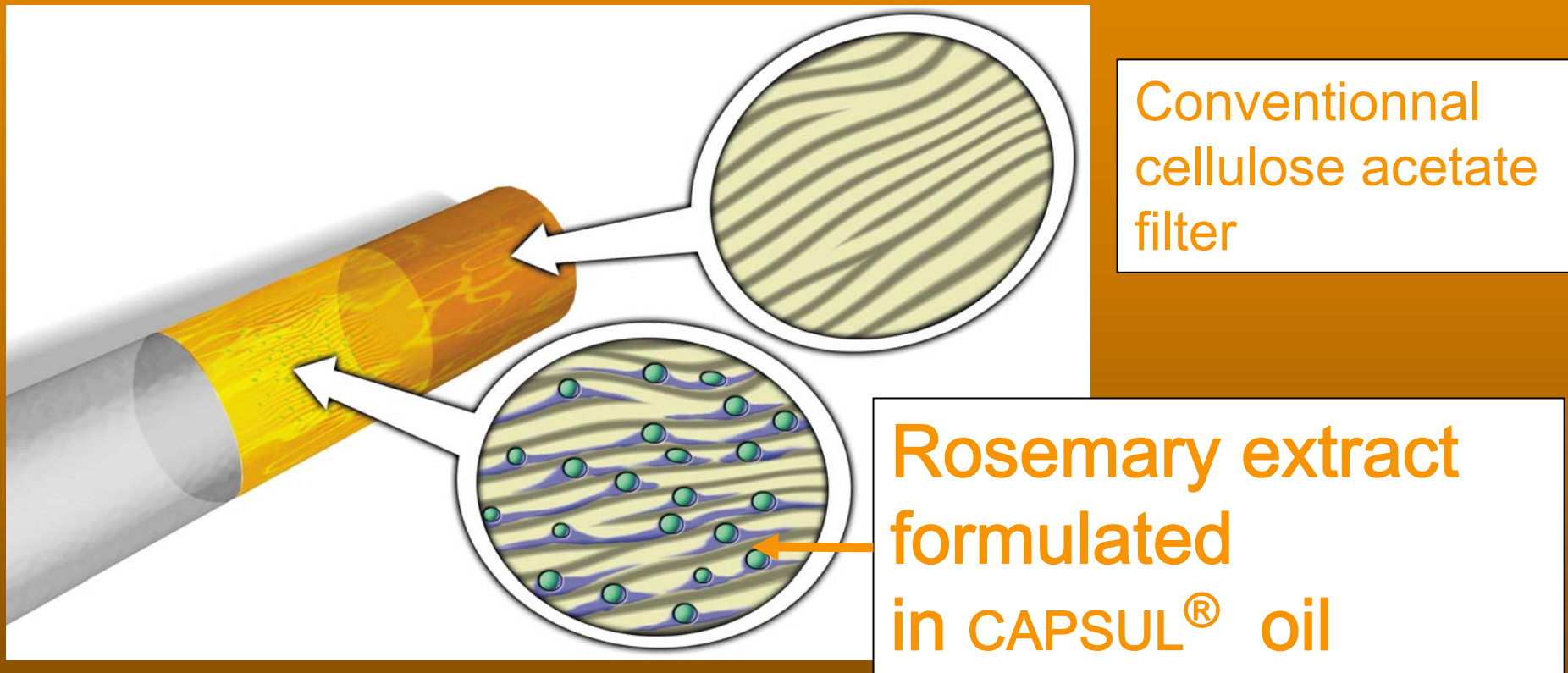
# Why removing free-radicals in cigarette smoke?

Reactive Oxygen Species (ROS) generated by free-radicals present in cigarette smoke induces an oxidative stress specially in the lungs.

Oxidative stress is involved in most diseases related to ageing particularly cancer and Cardiovascular diseases

Each cigarette smoke contains ca  $10^{17}$  free radicals!  
As free radicals are involved in chain reactions more than  $10^{20}$  strategic biological molecules may be damaged.

# New active filter based on Rosemary extracts

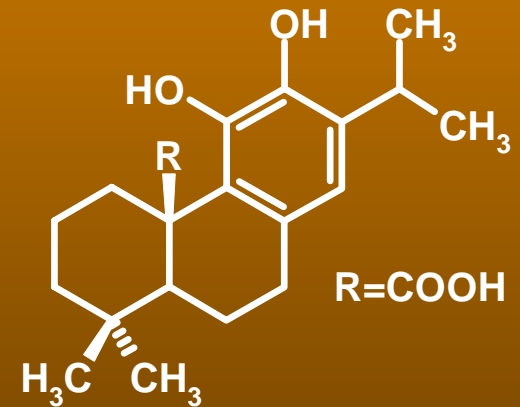
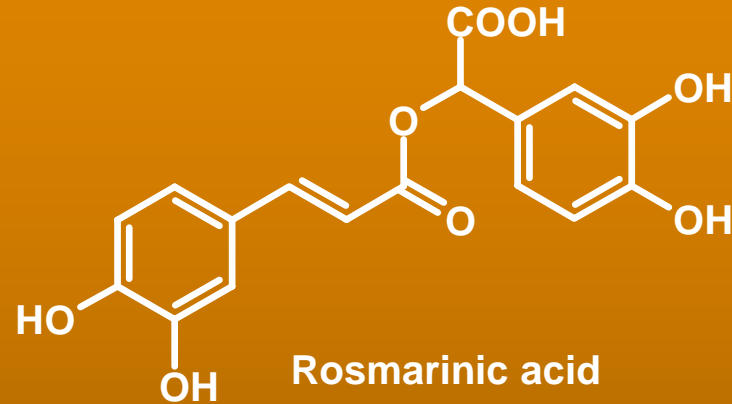


Iman EMAMI, Utilisation de composés polyphénoliques ou de leurs dérivés comme capteurs de radicaux libres dans les filtres de cigarette, Brevet européen WO9933365, 08.07.1999.

# Active molecule of *Rosemary* extract



*Rosemarinus*  
*Officinalis* L



Carnosic acid

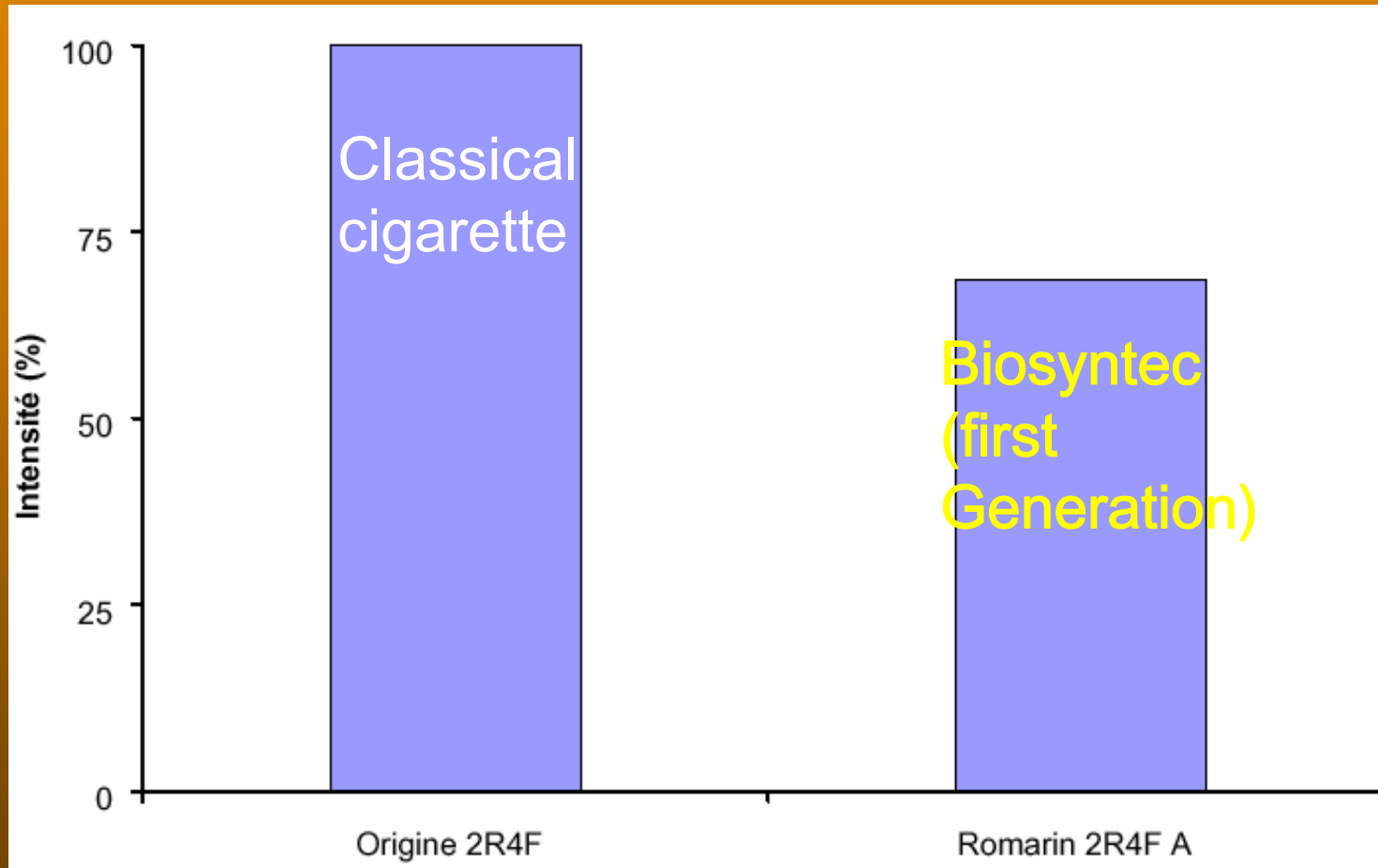
Biosyntec extraction process gives a *Rosemary* extract with a higher percent of active compounds.

# Identification of free radicals by LC/MS by the new method developed for this project

ESR (Pryor <i>et al</i> )	ESR (Kodama <i>et al</i> )	LC-ESI-MS/MS (this work)
Alkoxy $\text{RO}^\circ$ ( $\text{R} = \text{NO}_2$ isoprene)	$\text{OH}^\circ$	$\text{OH}^\circ$ , $\text{NO}_2^\circ$ , $\text{CH}_3^\circ$ , $\text{CN}^\circ$ , <b>Butadiene-<math>\text{H}^\circ</math>,</b> <b>Isoprène-<math>\text{H}^\circ</math></b>

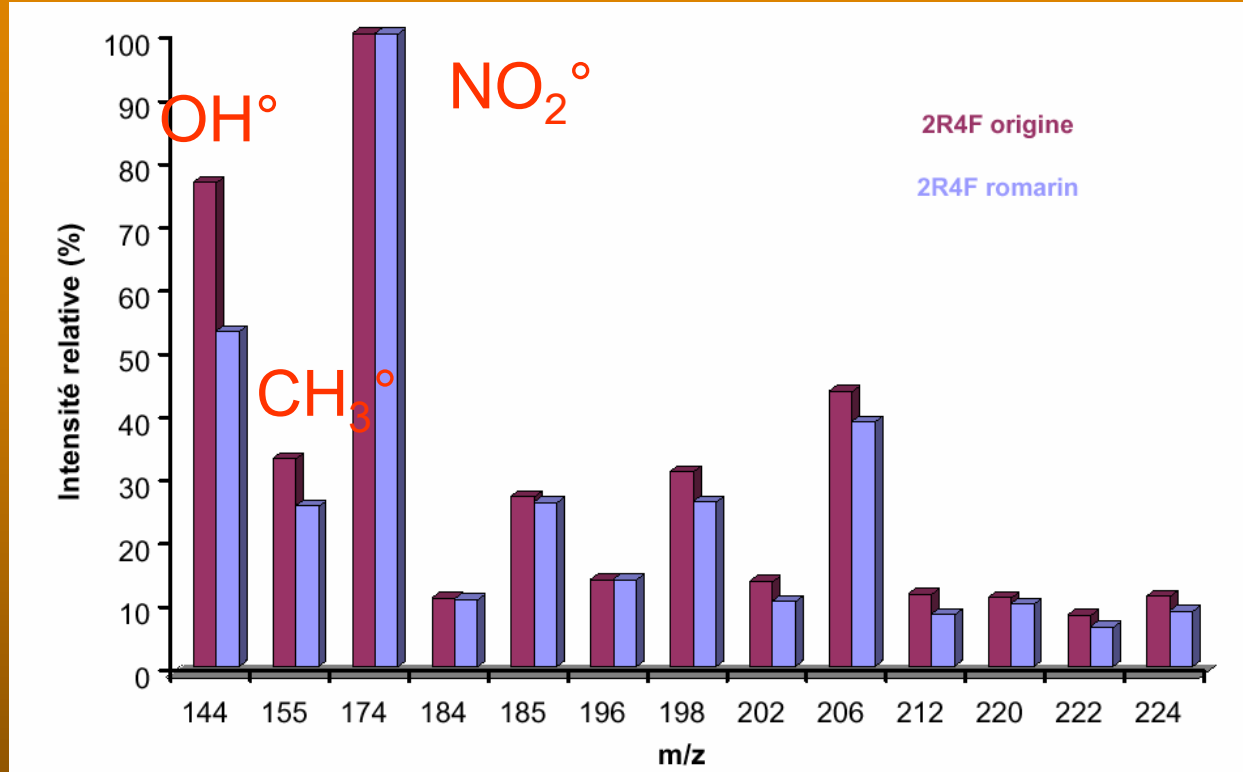
Many new free radicals in cigarette smoke have been detected

# Quantification of the $\text{OH}^\circ$ radical by LC/MS by the new method developed for this project



The  $\text{OH}^\circ$  amount may be reduced up to without affecting the smoking parameters, particularly the pressure drop.

# Open question

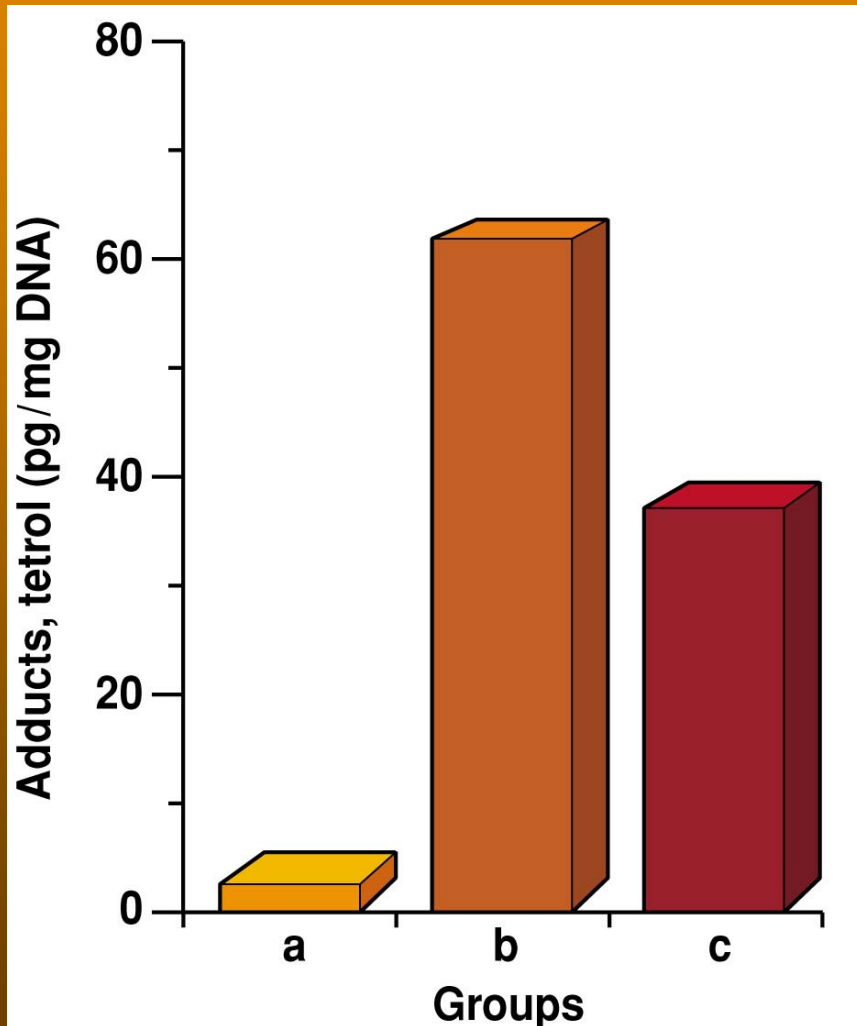


Our method shows that the different free radicals are removed with various efficiencies.

What is the toxicity of the O centered, N and C centered free radicals?



# DNA-adduct formation induced by cigarette smoke (cell free system)



Groups

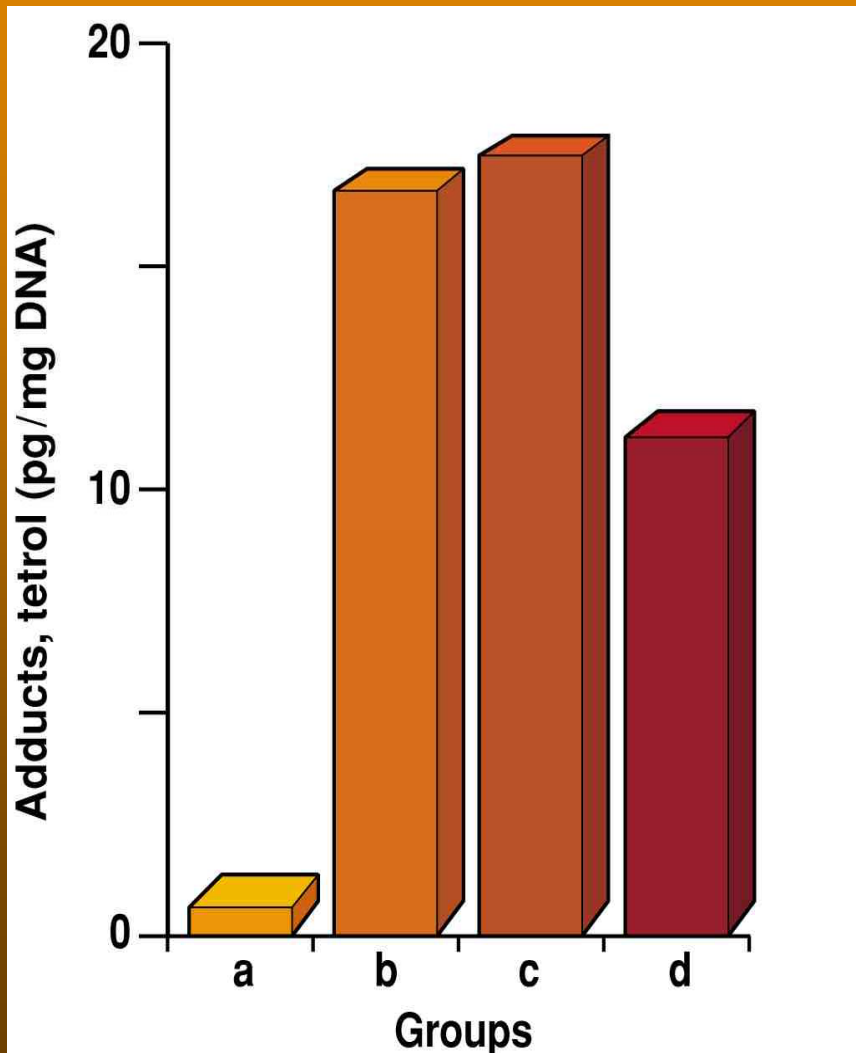
a B[a]P-7,8 diol alone

*+ cigarette smoke and tars*

b B[a]P-7,8 diol -Commercial filter

c B[a]P-7,8 diol -Biosyntec filter

# DNA-adduct formation induced by cigarette smoke in tumoral MCF-7 cells



Groups

a Blank

b B[*a*]P alone

*+ cigarette smoke and tars*

c B[*a*]P - Commercial filter

d B[*a*]P - Biosyntec filter

# Inhibition of adduct formation by cytochrome P450 and peroxidase pathway on MCF-7 line cells (high P450, low peroxidase)

Groups

Cytochrome P450

a Blank

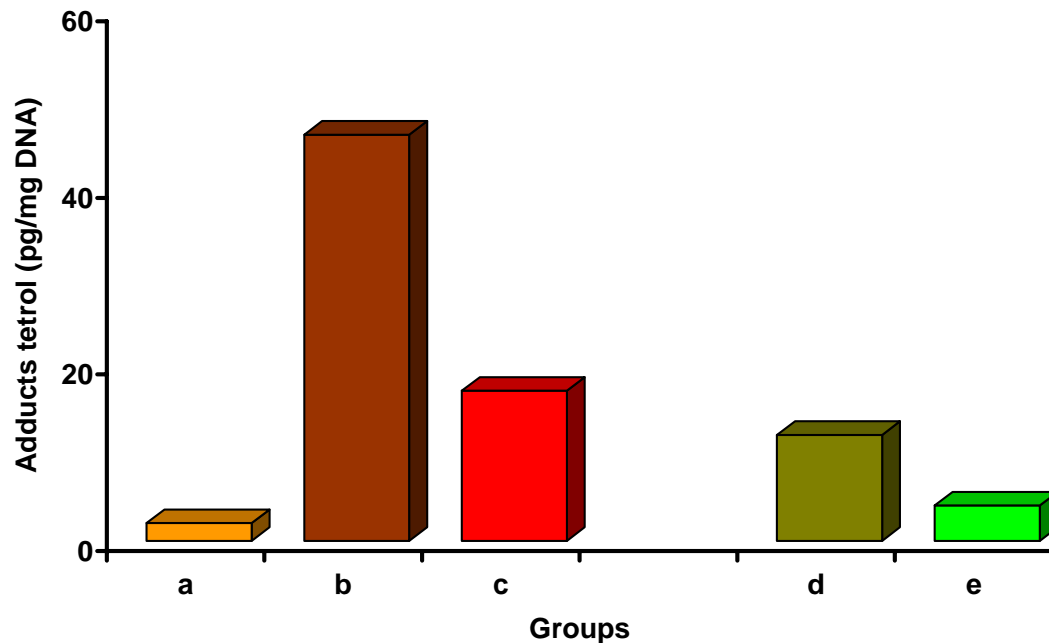
b Conventional filter

c Biosyntec filter

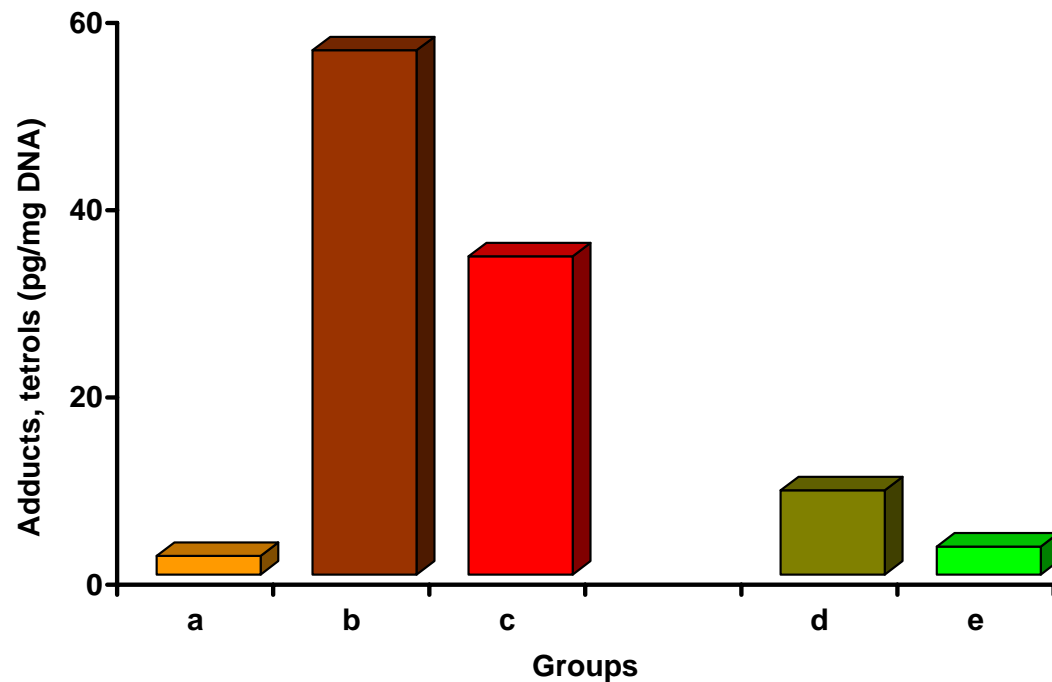
Peroxidase

d Conventional filter

e Biosyntec filter



# Inhibition of adduct formation by cytochrome P450 and peroxidase pathway on LH-60 line cells (low P450, high peroxidase)



Groups

Cytochrome P450

a Blank

b Conventional filter

c Biosyntec filter

Peroxidase

d Conventional filter

e Biosyntec filter

## Biological studies conclusion

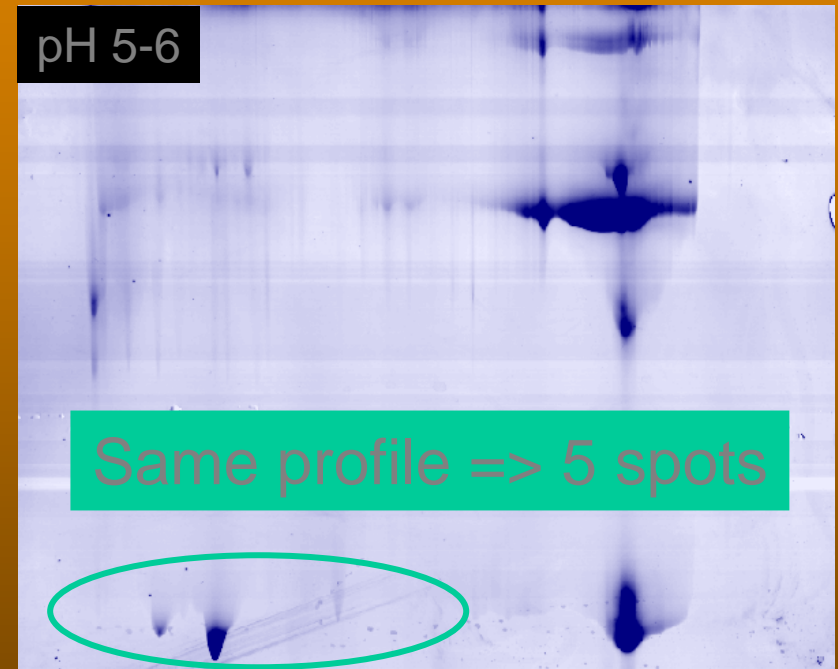
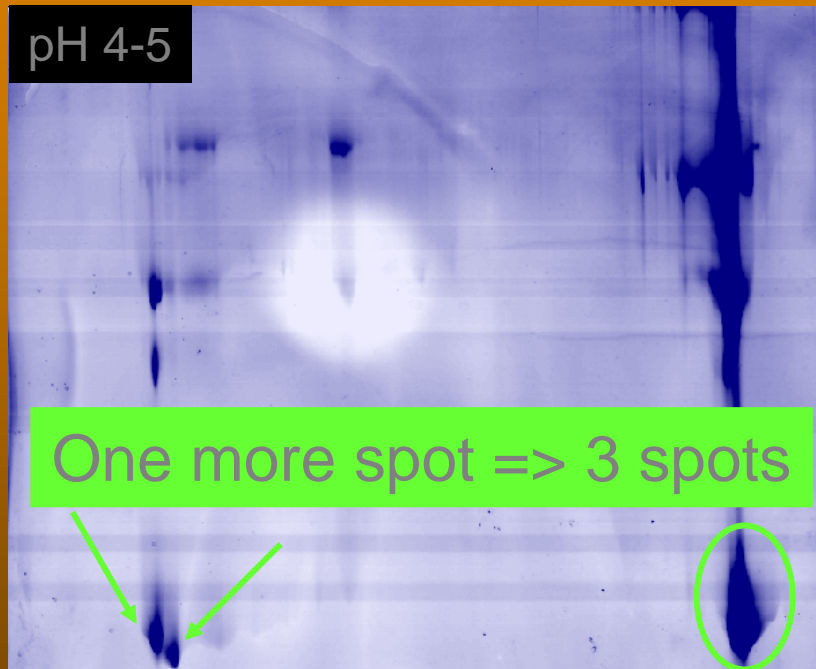
There is a *synergic* effect between Carcinogens contained in cigarette smoke tar like Benzo[*a*]Pyrene (B[*a*]P) and Reactive Oxygen Species (ROS) generated by free-radicals present in cigarette smoke.

Removing the free radicals is thus **more efficient** than expected on DNA adduct reduction!

# New methodology for characterizing oxidized protein in the plasma of smoking subject

## Analysis of apolipoprotein A1 from diabetic subjects

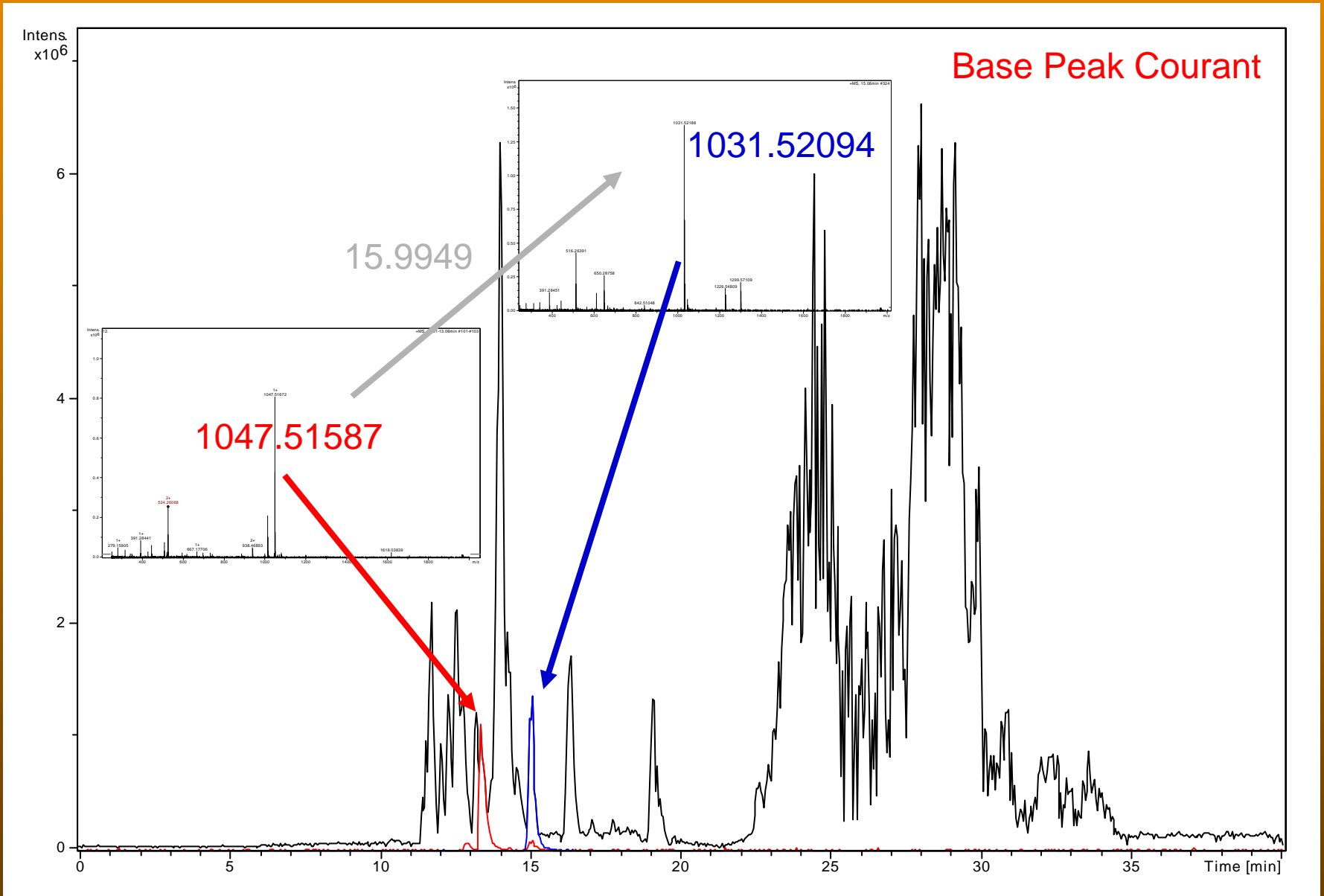
=> 2D gel electrophoresis of human plasma from diabetic subject



=> 8 apolipoprotein isoforms in diabetes pathology  
(only 5 in normal plasma)

The same technology is applied to the plasma of smokers

# Analysis of apolipoprotein A1 on Nano-LC-FT MSMS



⇒ Oxydation of peptides (exact mass)

# Next Step: Clinical Trial Protocol

Objective: determine the variation of DNA adducts on smokers using cigarette equipped by Biosyntech filters.

Number of volunteers: 90

Duration: one year

50 Biosyntec filter (20-30 per day),

20 smokers conventional cigarettes (20-30 per day)

20 smokers stopping just before the test (10 GSTM<sup>+</sup>, 10 GSTM<sup>-</sup>)

Medical control (months 0 « inclusion » and 12 « exclusion »)

Analysis months 0, 3, 6, 8 and 12

DNA adducts measurement (blood)

Oxidized proteins and lipids (new methodology)

8-hydroxy-deoxy-Guanosine (urine)

Genotyping of GSTM1



# Conclusion

**From our study on filters which efficiently remove free radicals from cigarette smoke we can conclude that “Evaluation of the risk reduction associated with smoking cigarettes” requires:**

- innovative technology for reducing the risk**
- new methodologies for both chemical and biological quantitation of the effect**
- clinical trial should be the last step**

# SUMMARY

- ⇒ Biosyntec new filters are based only on natural products already widely used in the food industry (Rosemary extract and CRODAMOL<sup>®</sup> oil for formulation).
- ⇒ Biosyntec extraction process gives a *Rosemary* extract with a higher percent of active compounds.
- ⇒ Biosyntec filters scavenge more than 30 % of free-radicals in cigarette smoke without modifying smoking conditions (pressure drop, tar weight). Furthermore, preliminary tests show that organoleptic properties of cigarette brands are preserved.
- ⇒ *In vitro* biological assays on DNA-adduct formation show results in agreement or better than the free-radical decrease.
- ⇒ A cohort of 90 volunteers has been recruited for clinical tests

# Biological studies summary.

⇒ DNA adduct formation from Benzo[*a*]pyrene is activated by cigarette smoke and tars.

⇒ Cell free experiments show that the Biosyntec filters decrease the amount of the adduct formation roughly in the proportion of the free-radicals decrease.

⇒ In MCF-7 tumoral cell line tobacco smoke from Biosyntec filters led to a rather unexpected protective effect against adduct formation.

⇒ Comparison of MCF-7 and LH-60 lines shows that both cytochrome P450 and peroxidase activation of B[*a*]P are reduced by Biosyntec filters