



Influence of the origin of the cigarette paper cellulose on the chemical composition of the smoke and « in vitro » toxicity testing on mainstream cigarette smoke

V. Troude¹, S. Achard¹, S. Destruhaut¹, C. Haond¹, J. Sarabia².

1. Altadis – Research Centre, 4 rue André DESSAUX, 45404 Fleury-les-Aubrais, France.

2. Altadis, Eloy Gonzalo 10, 28010 Madrid, Spain.

Cellulose fibre?

Wood versus...



Abaca



...Flax (*Linum usitatissimum*)



Esparto



Sisal



Hemp

not aroma and taste
analytical results consequences

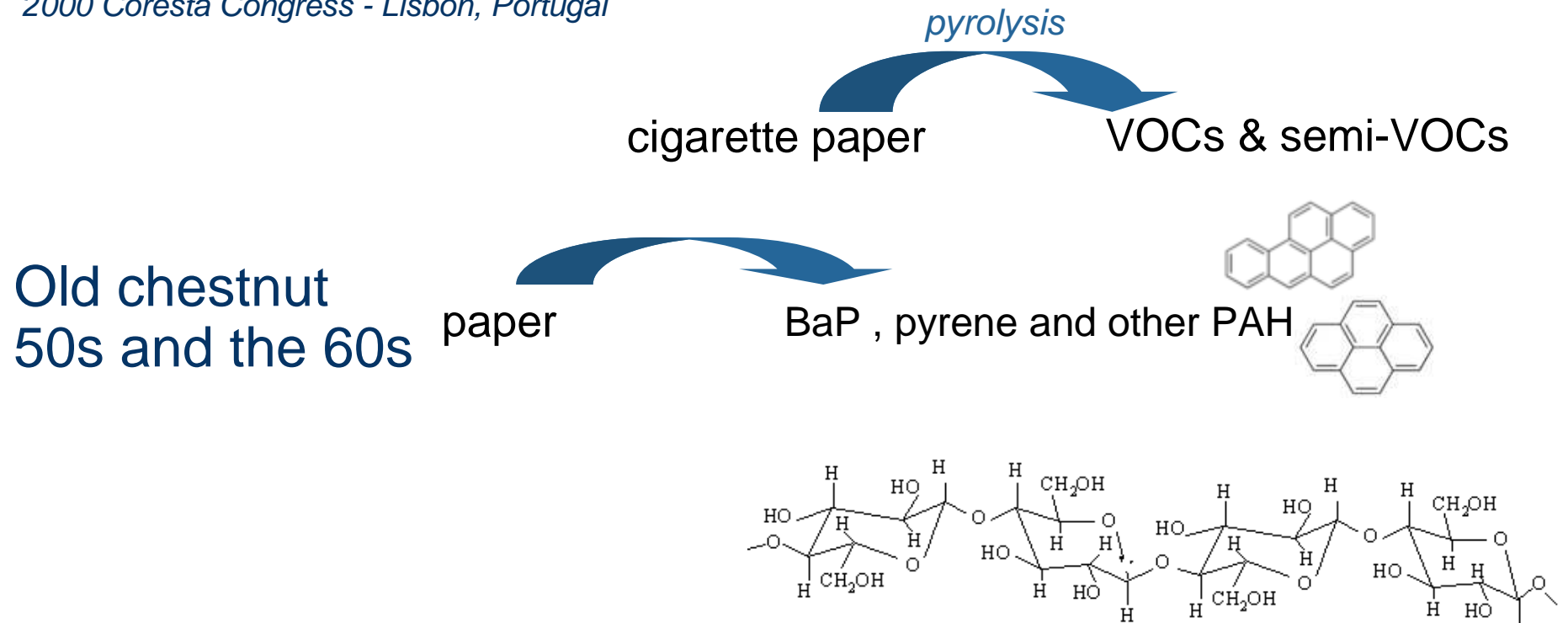
Analytic design?

1. Does the pyrolysis of the paper alone generate toxic compounds?
2. Are the products generated by pyrolysis of paper different according to the origin of the cellulose?
3. Are the mainstream smoke properties modified depending on the origin of the cellulose paper?

Background

“The Comparative Analysis Of Smoke Components Delivered From Old chesnut Cigarette Papers Manufactured By Flax And Wood Pulp”

*Jong-Yeol Kim, et al. Korea Ginseng and Tobacco Research Institute
2000 Coresta Congress - Lisbon, Portugal*



wood vs. flax paper % lignin - hemicellulose - cellulose

Restricted design

- Two papers : mix flax-hemp-esparto vs. pure wood paper
- Manufactured cigarettes

ProductA-0 and ProductA-100

ProductB-0 and ProductB-100.

The number stands for the percentage of wood paper.

- Chemical evaluation
- Biological Activity assessments

■ Chemical evaluation

- **Hoffmann list** Except the pH level and Trace metals (35 constituents)
- Benzo(a)Pyrene completed with the evaluation of Pyrene and Dibenzopyrenes.

■ Biological Activity assessments

→ A Bacterial Mutagenicity assay:

- the Ames test

→ a Mammalian cell Cytotoxicity assay:

- the Neutral Red Uptake test

→ a Mammalian cell Genotoxicity assay:

- the MicroNucleus assay

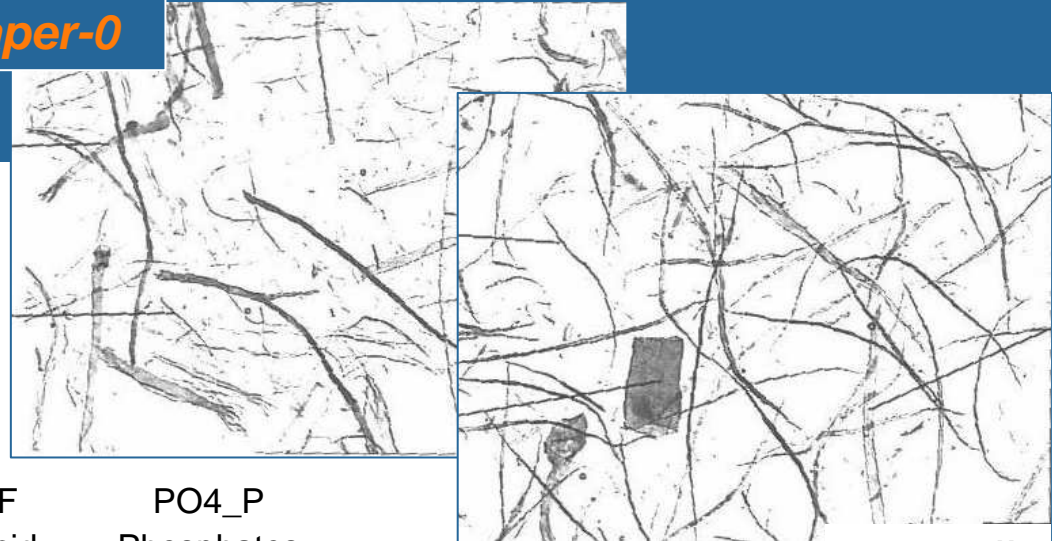
tests on TPM

completed with vapor phase analysis

■ Biochemical assay "GSH depletion"

- test to rapidly assess the radical profile of the vapor phase.

Paper-0



Paper-100

■ Paper analysis

- Fiber composition : ISO 9184
- Essence : optical technique

	CA_CO3_P Calcium_Carbonate	CIP_CF Citric_acid	PO4_P Phosphates
Paper-0	28,10	0,07	0,04
Paper-100	22,50	0,13	0,00

■ Physical analysis

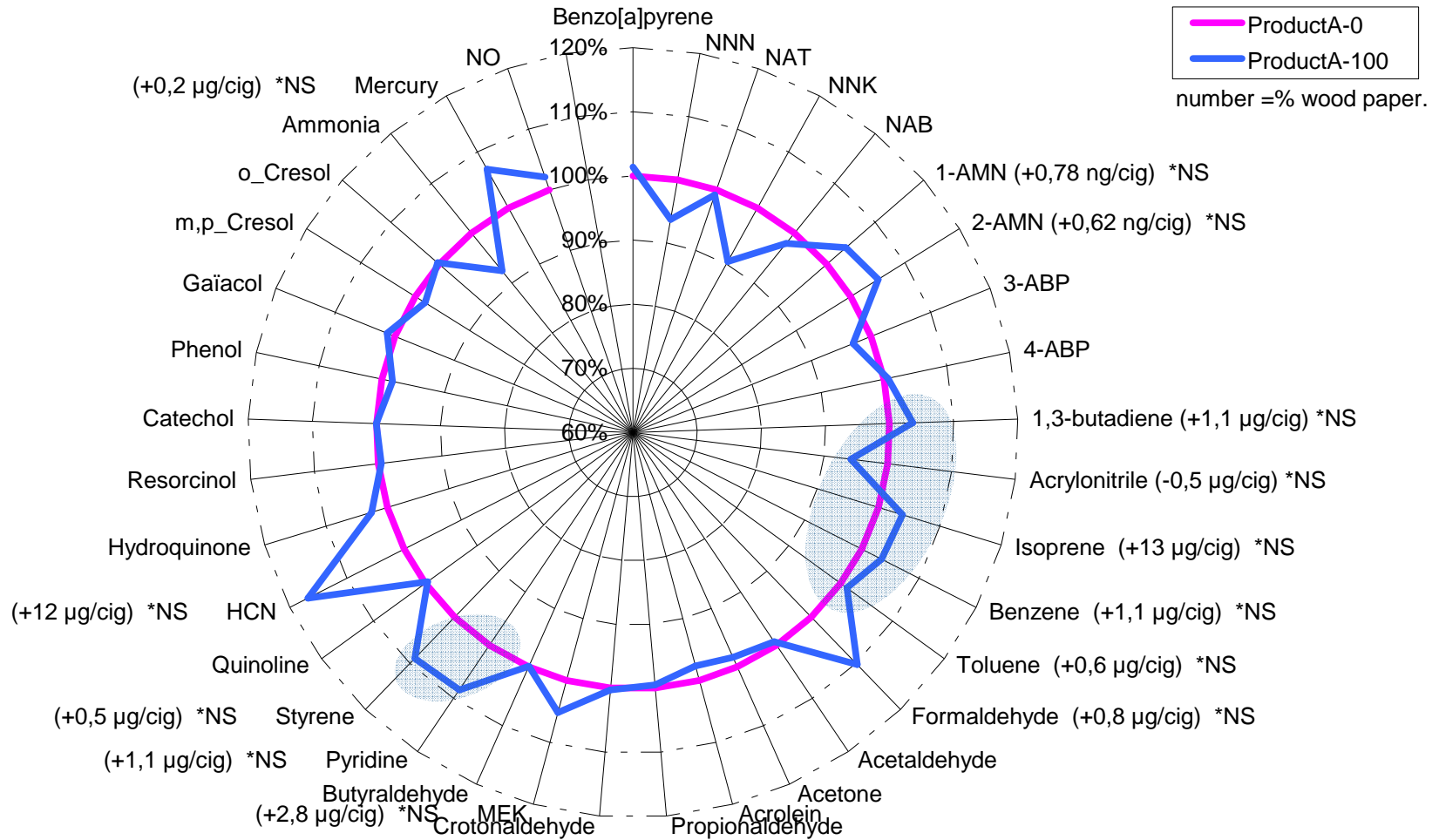
- Sampling manufactured cigarette : ISO 8243:2003

	WCIG	WNTM	WTOBC	TOBCL	DIAM	PD	PDEF	FV	HARD
ProductA-0	920	206	704	62,7	7,87	80	93	17,5	98
ProductA-100	904	210	686	62,6	7,87	79	91	17	94
ProductB-0	1002	215	774	62,7	7,91	105	125	22	154
ProductB-100	987	216	757	63,1	7,9	103	123	22,1	148

Hoffmann yields

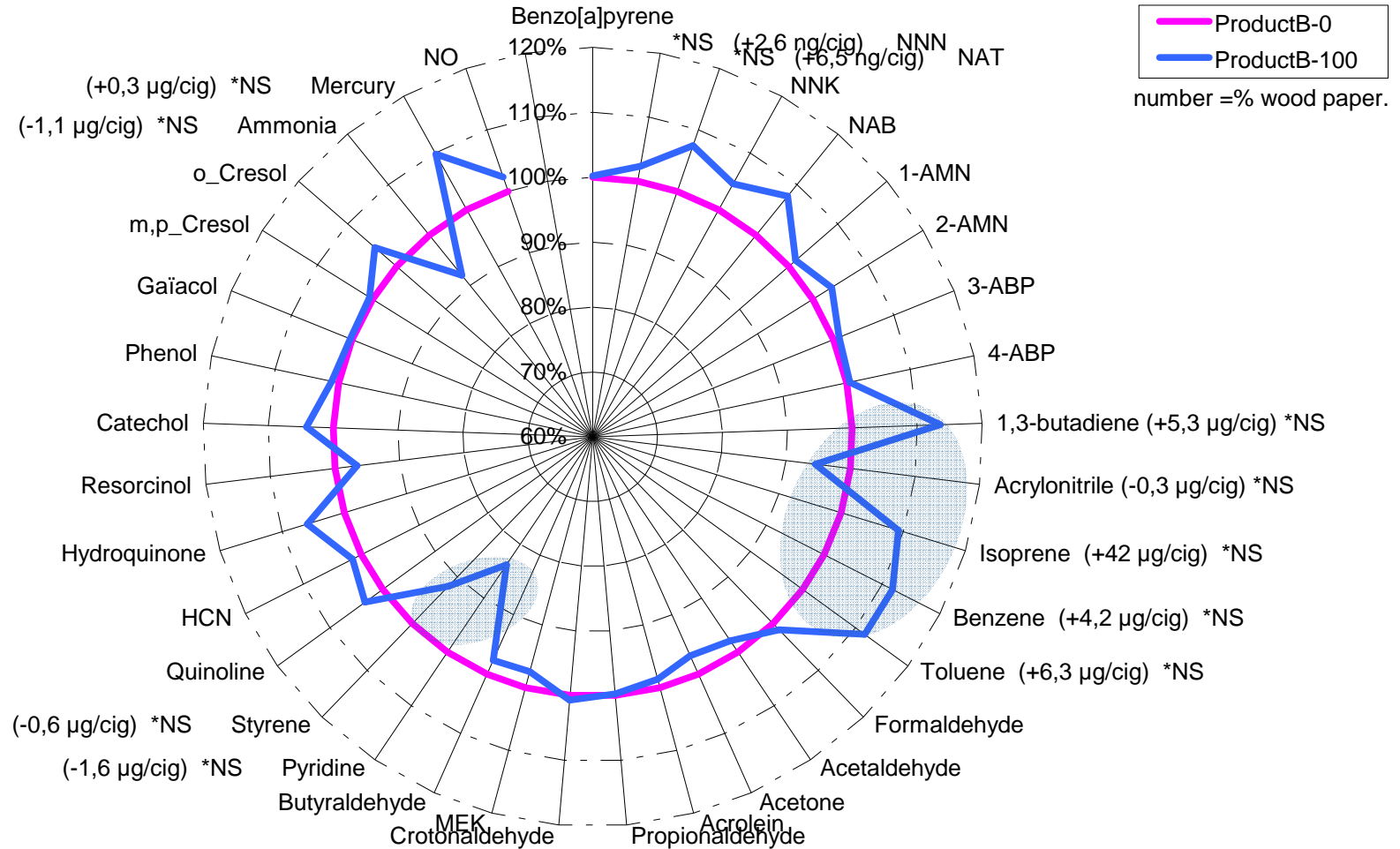
- BaP : ISO/CD 22634
- “Hoffmann analytes”: in-house validated methods.
 - TSNA LC-MSMS.
 - Aromatic Amines GC/MS (methane).
 - Volatiles cryogenic traps & GC/FID.
 - Carbonyls HPLC-UV
 - Semi-Volatiles cryogenic traps & GC/FID.
 - Hydrogen Cyanide automated continuous flow analyzer
 - Phenolics HPLC-fluo
 - Ammonia IC
 - Mercury AA
 - NO puff by puff -chemiluminescent emission
- Smoking regime : ISO conditions
- Delivery time : 1 month
- N = 316 analytic results
- Average with confidence limit at 95%

Hoffmann yields /1



no significant difference
between ProductA-100 and ProductA-0.

Hoffmann yields /2



no significant difference
 between ProductB-100 and ProductB-0.

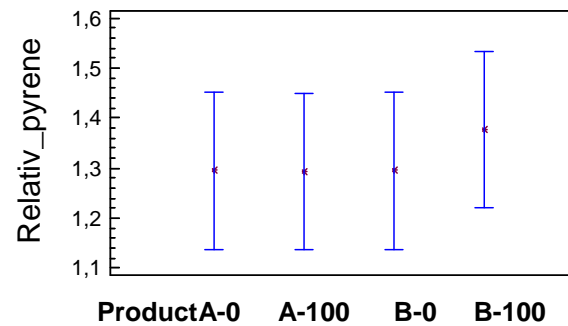
Pyrene and dibenzopyrene evaluation

Dibenzopyrene : 4 carcinogens were looking

- Dibenzo(a,e) pyrene,
- Dibenzo(a,i) pyrene,
- Dibenzo(a,h) pyrene,
- Dibenzo(a,l) pyrene.

Samples < LOD = 4 ng/cig

Moyennes et intervalles à 95,0% LSD



Analyse de variance pour Relativ_pyrene - Somme des carrés de type III

Source	Somme des carrés	Ddl	Carré moyen	F	Proba.
EFFETS PRINCIPAUX					
A:terme	0,02085	3	0,00695	0,18	0,9066
B:repetition	0,02235	3	0,00745	0,19	0,8978
RESIDU	0,3454	9	0,0383778		
TOTAL (CORRIGE)	0,3886	15			

Tous les F sont basés sur l'erreur résiduelle quadratique moyenne.

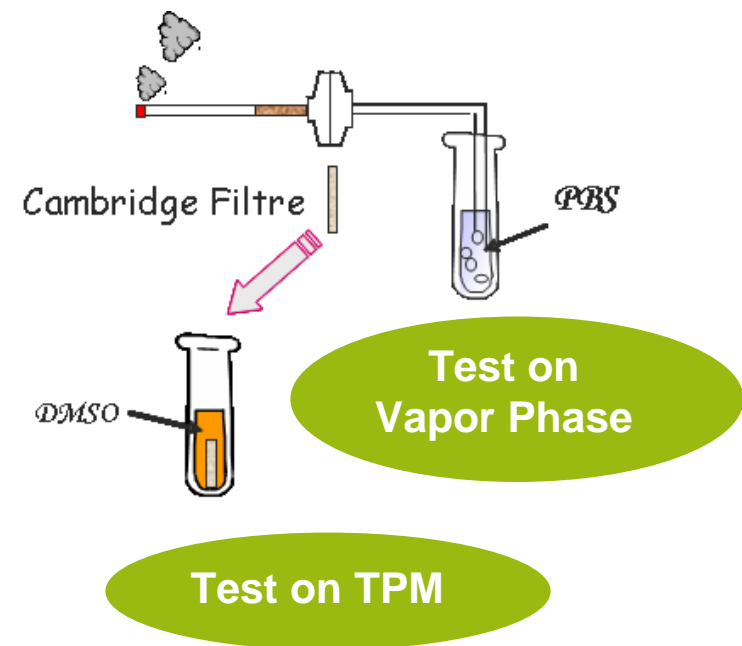
No statistical difference between the ProductB-0 and ProductB-100 and ProductA-0 and ProductA-100.

No statistical difference between the Wood paper and Flax paper

Biological activity assessments

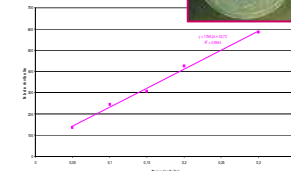
- Sample generation for the recommended in vitro testing
 - Same for all the biological assays (Mutagenicity test, Cytotoxicity and Genotoxicity assays).
 - Environmental conditions & ISO smoking conditions.
 - SM450 linear smoking machine

- Vapor phase
 - KC5 linear smoking machine



TPM

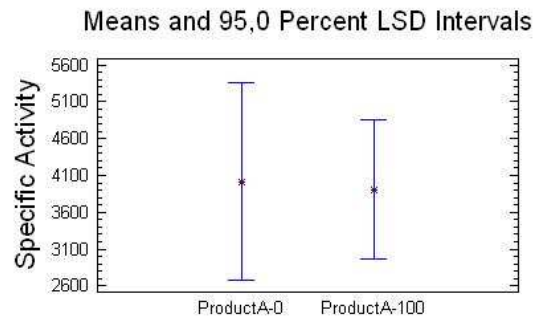
Mutagenicity
Ames test



Mutagenic activity- Ames test

Salmonella typhimurium TA98 (+S9)
72 hours-incubation

ProductA



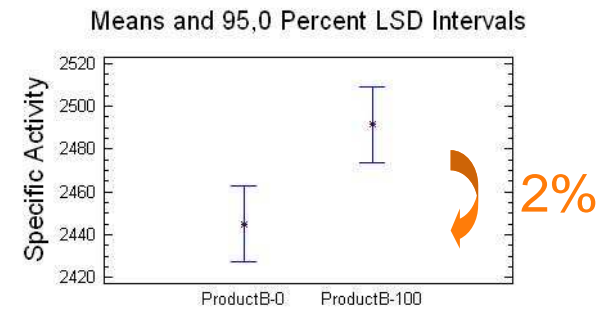
Multiple Range Tests for Specific Activity by Paper_level

Method: 95,0 percent Student-Newman-Keuls

Paper_level	Count	Mean	Homogeneous Groups
ProductA-100	6	3910,0	X
ProductA-0	5	3950,0	X

No statistical difference between the ProductA-0 and ProductA-100.

ProductB



Multiple Range Tests for Specific Activity by Paper_level

Method: 95,0 percent Student-Newman-Keuls

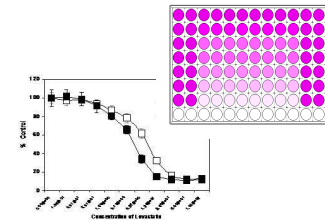
Paper_level	Count	LS Mean	LS Sigma	Homogeneous Groups
ProductB-0	6	2445,0	5,84166	X
ProductB-100	6	2491,33	8,84166	X

Not relevant for biological response
Mutagenicity of ProductB condensate
unmodified with the paper type

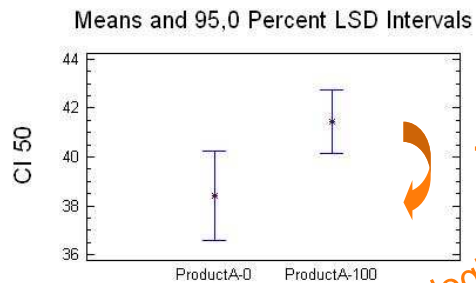
Cytotoxic activity- Neutral Red Uptake Test

Chinese Hamster Ovary cells (-S9)
24 hours-incubation

Cytotoxicity Neutral Red Uptake test



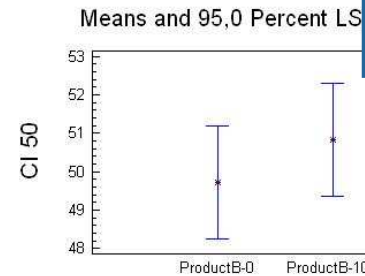
ProductA



7%

TPM

ProductB



Multiple Range Tests for CI 50 by Paper type

Method: 95,0 percent Student-Newman-Keuls

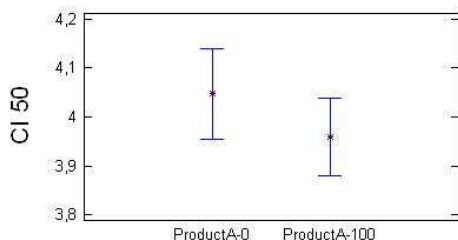
paper type	Count	LS Mean	LS Sigma	Homogen Group
ProductA-0	10	38,412	1,19509	X
ProductA-100	12	41,4401	0,845058	X

Multiple Range Tests for CI 50 by Paper type

Method: 95,0 percent Student-Newman-Keuls

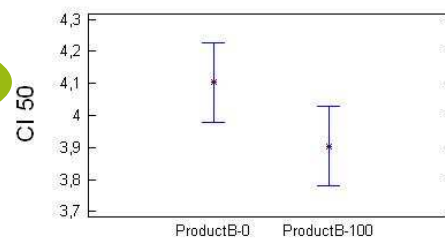
Paper type	Count	LS Mean	LS Sigma	Homogeneous
ProductB-0	12	49,7185	0,969691	X
ProductB-100	12	50,8196	0,969691	X

Means and 95,0 Percent LSD Intervals



Vapor Phase

Means and 95,0 Percent LSD Intervals



Method: 95,0 percent Student-Newman-Keuls

Paper type	Count	LS Mean	LS Sigma	Homogeneous Groups
ProductA-100	6	3,959	0,0441188	X
ProductA-0	5	4,04733	0,050944	X

Method: 95,0 percent Student-Newman-Keuls

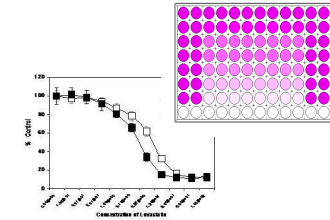
Paper type	Count	LS Mean	LS Sigma	Homogeneous Groups
ProductB-100	6	3,90433	0,0	X
ProductB-0	6	4,10383	0,0	X

Cytotoxic activity- Neutral Red Uptake Test

Chinese Hamster Ovary cells (-S9)

24 hours-incubation

Cytotoxicity Neutral Red Uptake test

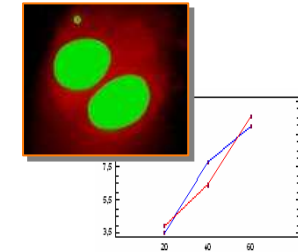


Whatever the product, the cytotoxicity of both
Condensate and Vapor Phase
was unmodified with the paper type.

Genotoxic activity- Micronucleus assay

Chinese Hamster Ovary cells

24 hours-continuous exposure (-S9)



➔ *Genotoxic assessment*
% of Micronuclei/Binucleated cell

$\% \mu\text{Nyx in Bi} = 100 \cdot [\text{Number of MN counted in BiN} / \text{Number of BiN}]$.

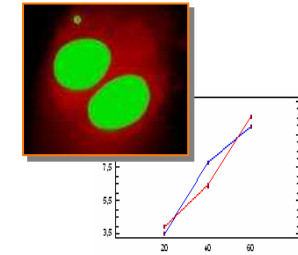
➔ *Toxicity evaluation*
Cytokinesis Block Proliferation Index (CBPI)

$\text{CBPI} = [\text{Mononucleated cells} + 2 \cdot \text{binucleated cells} + 3 \cdot \text{trinucleated cells} + 4 \cdot \dots] / \text{Total Cells}$

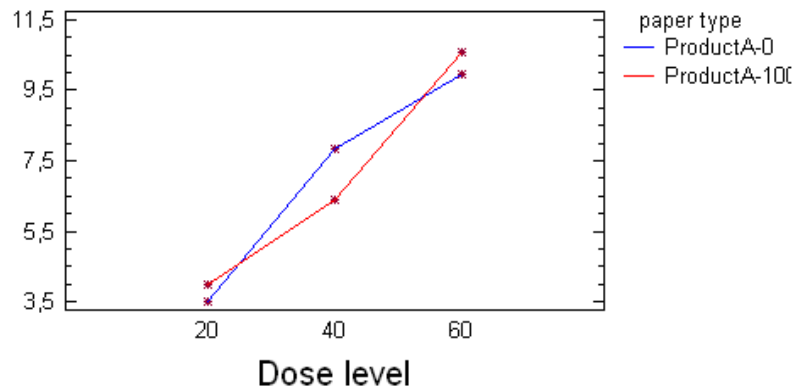
Genotoxic activity- Micronucleus assay

Only performed on ProductA-0 and A-100.

Comparative results dose per dose : 20, 40 & 60 µg TPM/ mL



%µNyx in Bi Interaction Plot



des étendues multiples pour %µNyx ds Bi par Dose

de: 95,0 % Newman-Keuls

Effectif	Moy. MC	Ect-types MC	Groupes homogènes
8	3,7625	0,787548	X
8	7,11125	0,787548	X
9	10,2422	0,804633	X

Analysis of Variance for %µNyx in Bi - Type III Sums of Squares

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
MAIN EFFECTS					
A:Dose level	127,029	2	63,5146	8,69	0,0021
B:paper type	0,287359	1	0,287359	0,04	0,8450
INTERACTIONS					
AB	9,41551	2	4,70776	0,64	0,5364
RESIDU	138,935	19	7,31237		
TOTAL (CORRIGE)	280,292	24			

All F-ratios are based on the residual mean square error.

No statistical difference between ProductA-0 and ProductA-100

Conclusion of the Biological Activity assessments

wood vs. flax paper

	<i>ProductA</i>	<i>ProductB</i>
Mutagenicity: Ames test	No difference between ProductA-0 and ProductA100	No difference between ProductB-0 and ProductB100
Cytotoxicity: Neutral Red test	No difference between ProductA-0 and ProductA100	No difference between ProductB-0 and ProductB100
Genotoxicity: MicroNucleus test	No difference between ProductA-0 and ProductA100	

Biological Activity unmodified with the paper type
for ProductA and for ProductB.

Biochemical assay "GSH depletion" test

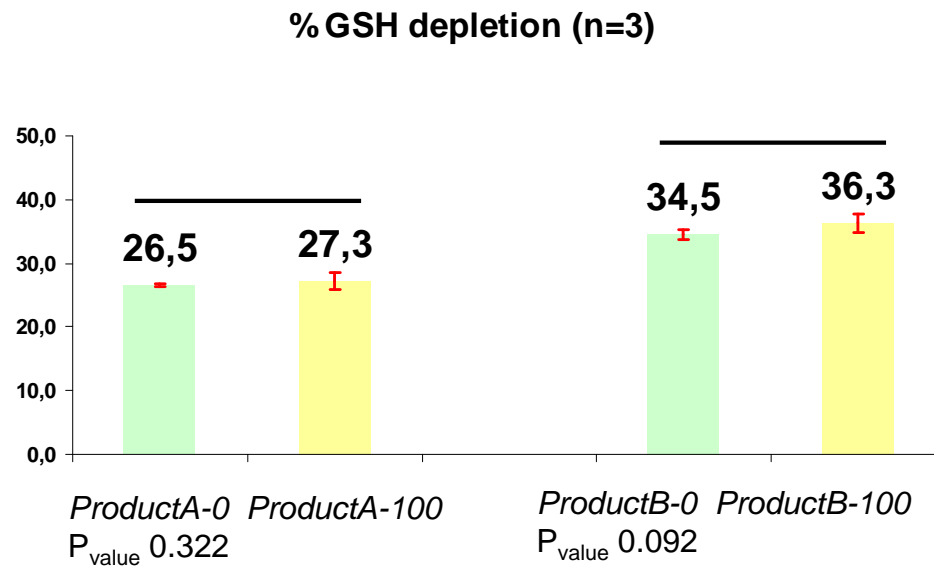
vapor phase



Biochemical assay
GSH depletion



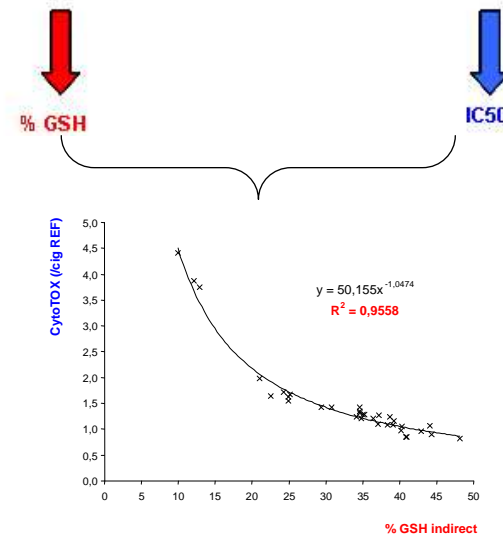
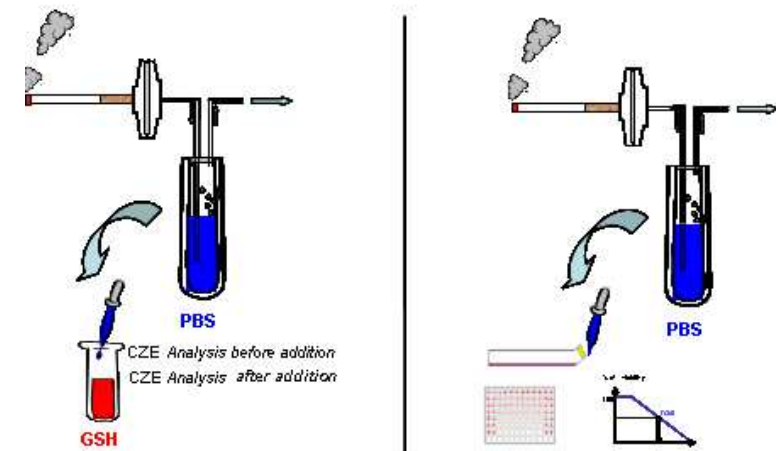
Cytotoxicity
Neutral Red test



ProductA

ProductB

Toxicity of the vapor phase unmodified by the paper type



2006 Coresta Congress - Paris, France

To conclude

- Question

Are the smoke properties modified depending on the origin the cellulose paper?

NO

아니요

- Neither chemical evaluation (based on the “Hoffmann list” compounds, Pyrene and Dibenzopyrenes) nor biological activity assessments (bacterial mutagenicity, mammalian cell cytotoxicity and genotoxicity assays)

reveal a significant difference between cigarettes manufactured with wood or flax paper

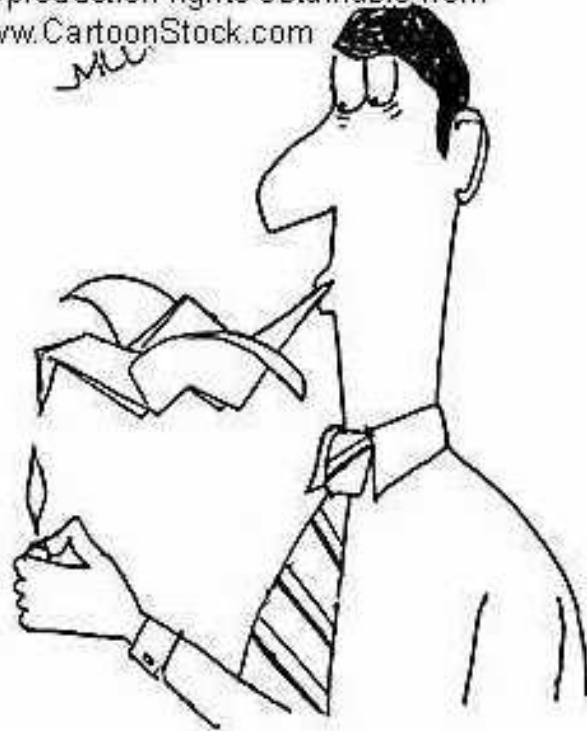
Acknowledgements

to the french & spanish team



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Enjoy your own cigarette paper.