60th TSRC Montréal, Québec Canada

HCN and tobacco precursors.

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Objective :

To develop an approach to evaluate effective contribution of tobacco nitrogen compounds on HCN delivery in mainstream smoke.

Structure:

#1 - Introduction
#2 - Methodology
#3 - Precursors list
#4 - Trial terms and smoking tests controls
#5 - Impact and contribution of precursors
#6 - Modeling
#7 - Conclusions

Methodology:

- **Spiking of potential precursors at different amounts on US blend prior to cigarette making. (NTM and tobacco weight = constant).**
- **HCN quantifications with classical method.**
- **Smoking tests with ISO conditions.**

Constraints:

- **Evaluation of precursors during cigarette smoke generation.**
- **The quantities added must be adapted according to the potential impact on HCN and solubility of each nitrogen compounds.**

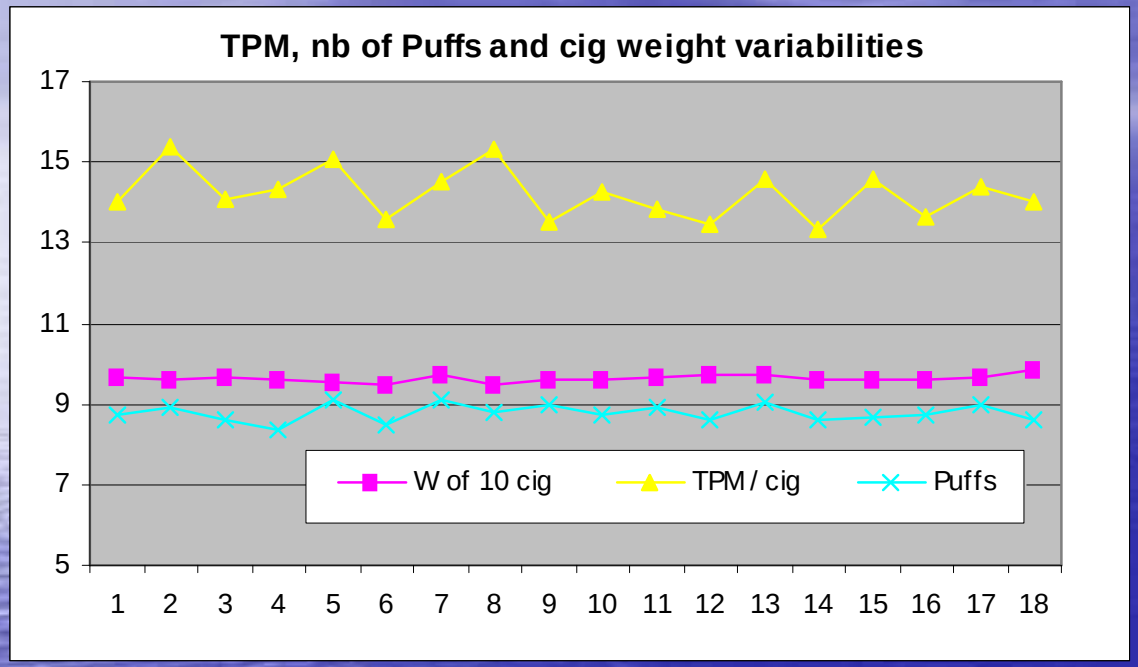
Precursors list:

Proteins: Albumin and γ Globulin.
Di-peptides: Ala - Ala and Phenyl Ala - Ala.
Amino-acids: ASN, ASP, PRO and GLN.

Miscellaneous:
- Tobacco pigments (Extracted from Air cured tobacco).
- Urea.

2, 3, 4 or 5 doses depending on availability and cost.

Smoking tests controls:



Smoke parameters report for albumin trial.

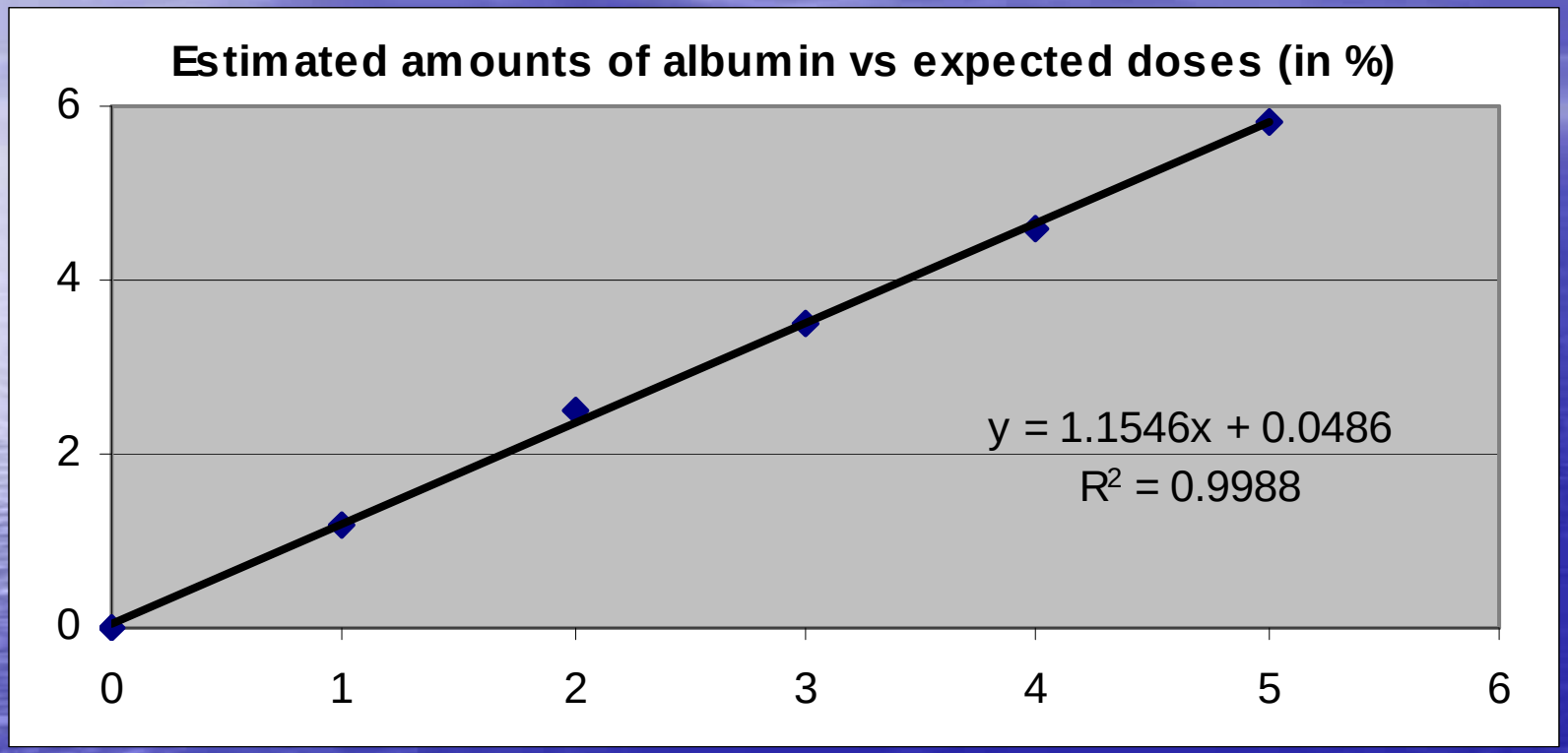
- No significant effect of doses on these responses.

Statistical report for control and trial terms:

- No significant effect of precursors on these responses.

		TPM / cig	Nb of Puff
Control	S.D.	0.55	0.19
Control	Av.	14.51	7.90
Control	CV.%	6.77	2.43
		TPM / cig	Nb of Puff
Trial	S.D.	1.06	0.22
Trial	Av.	14.25	8.78
Trial	CV.%	7.45	2.48

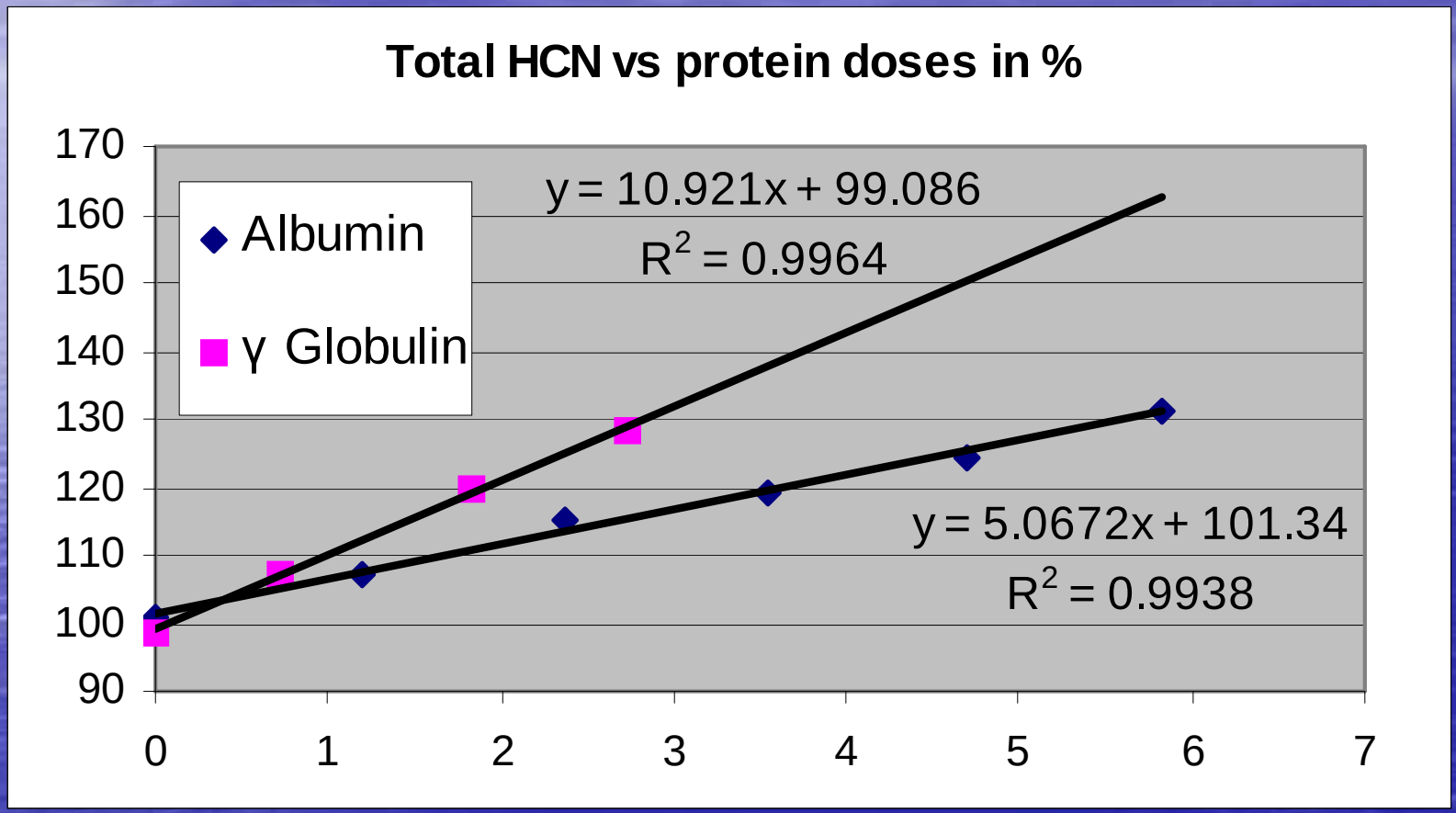
Enrichment controls:



Example: 5 doses of albumin, estimated by nitrogen gain.

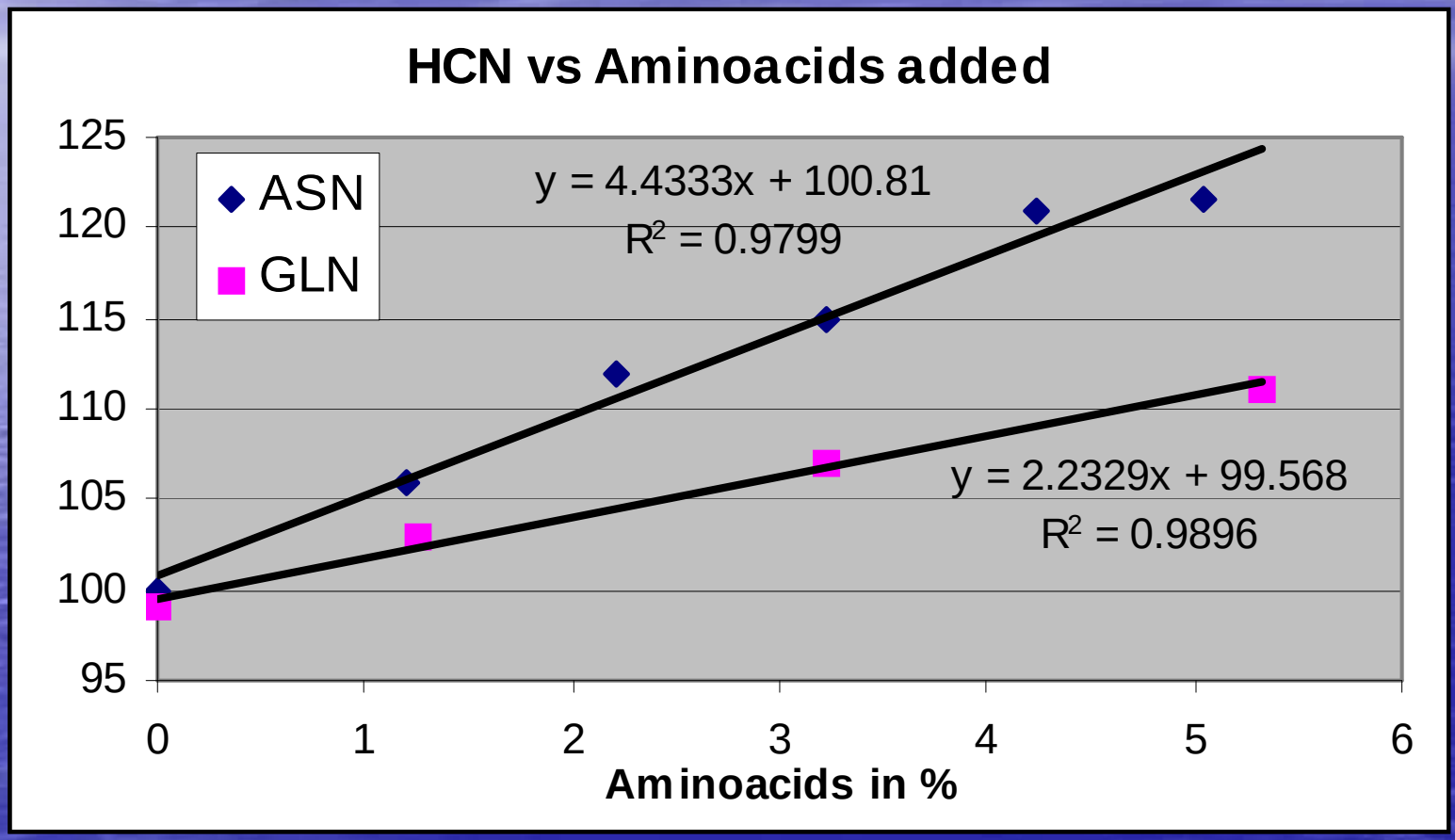
> Accordance between estimated and expected doses.

Protein influence on HCN in MSS



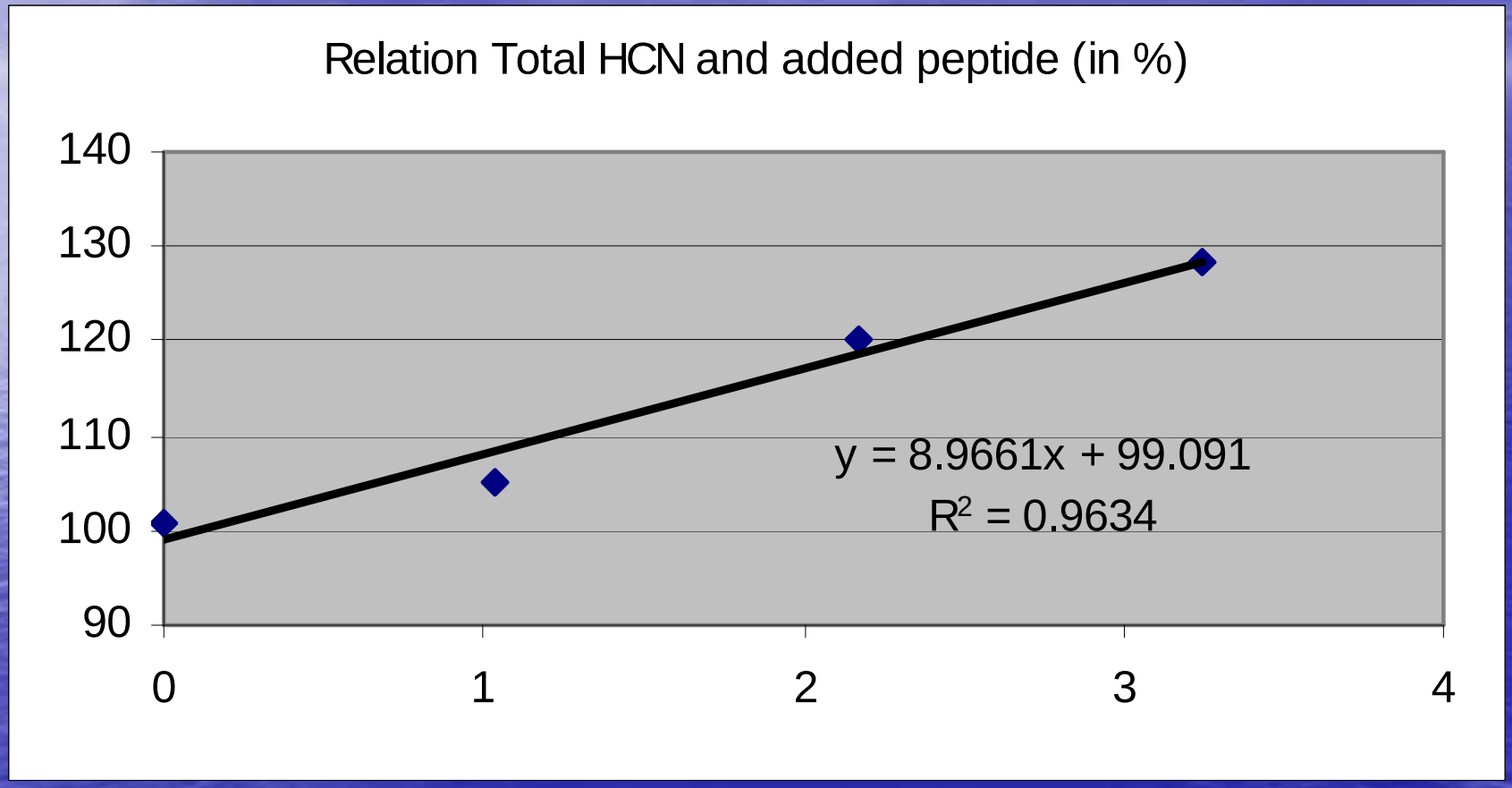
- Difference of contribution on HCN formation.

Amino acids impact on HCN:



- No significant incidence of proline and aspartic acid on HCN formation.

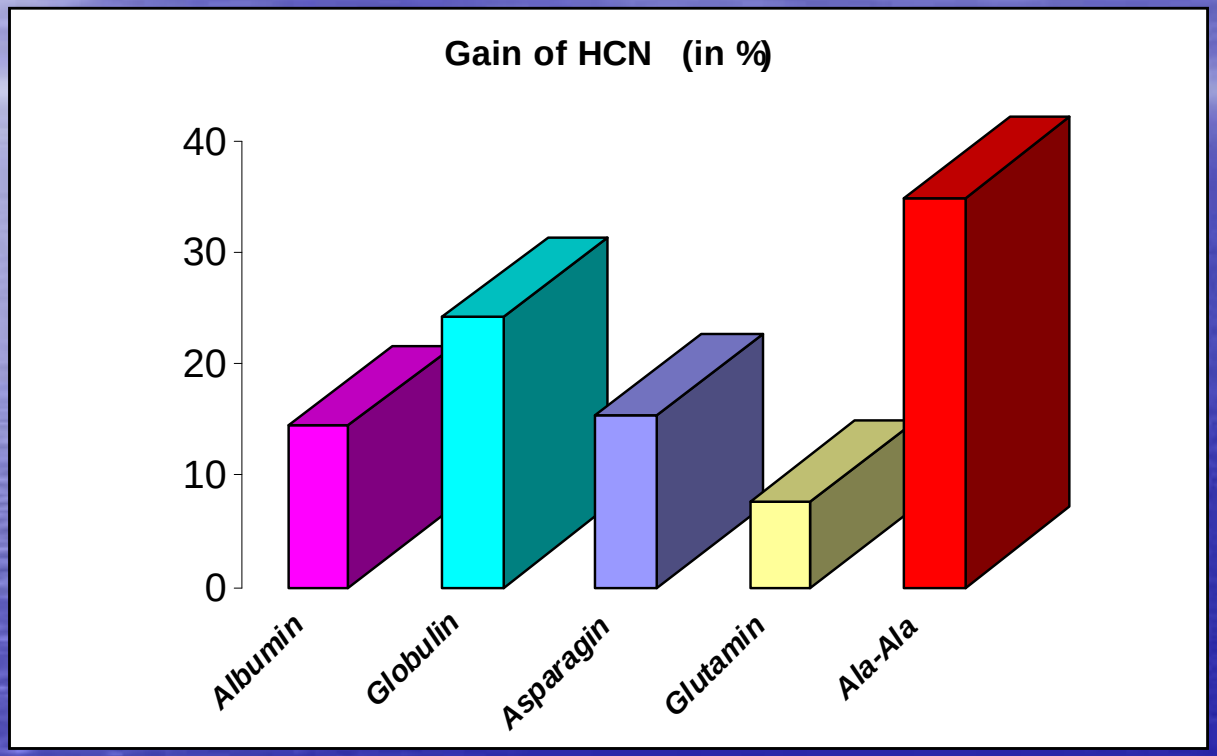
Polypeptide influence on HCN:



Example: ALA-ALA

Comparison of precursor contributions :

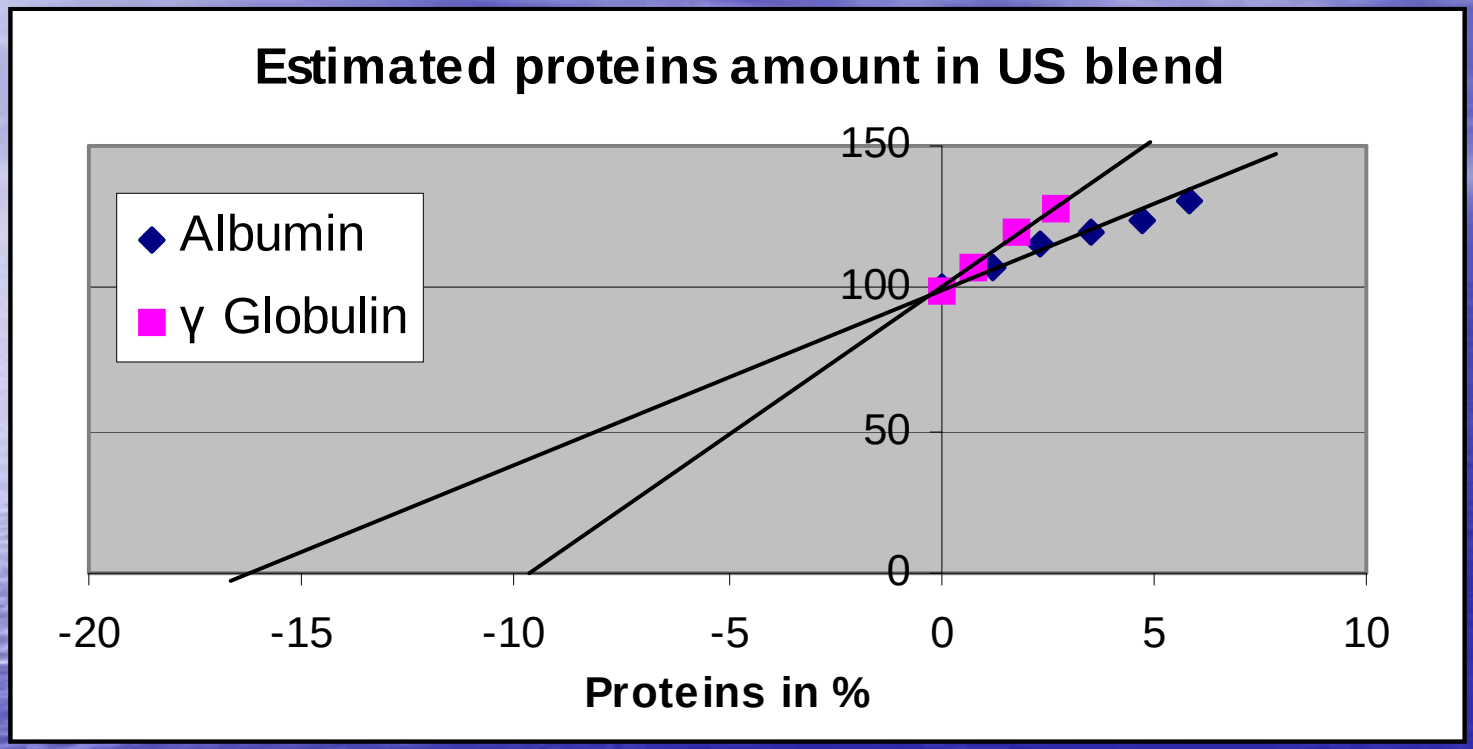
Calculated gain in HCN for 25 millimoles of precursors per 100 g of tob.



- **Confirms the differences of contribution on HCN for proteins or amino acids.**

- **No significant impact of tobacco pigments and urea on HCN formation.**

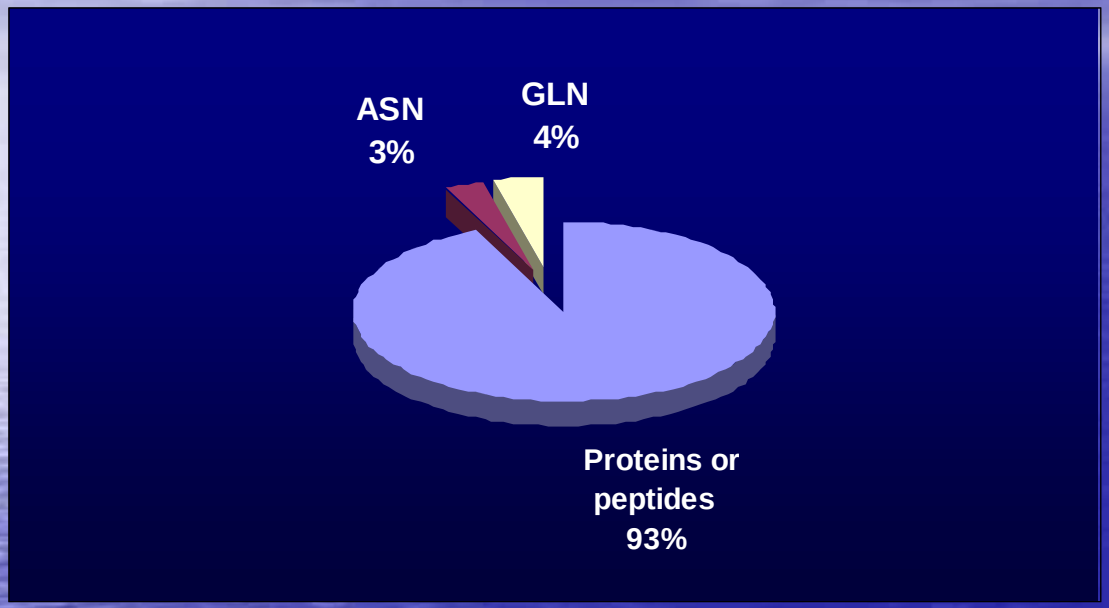
Precursors evaluation in no-enriched blend:



By using known addition approach, possible estimation of protein amounts.
In this blend: Between 10 and 16% of proteins >> Average: 13%.
(~1.7 % expressed in Nitrogen).

• Total HCN response of blend: Equivalent to ~11% of peptides or ~ 20% of amino acids.

Relative contribution of precursors for no-enriched blended cigarette:



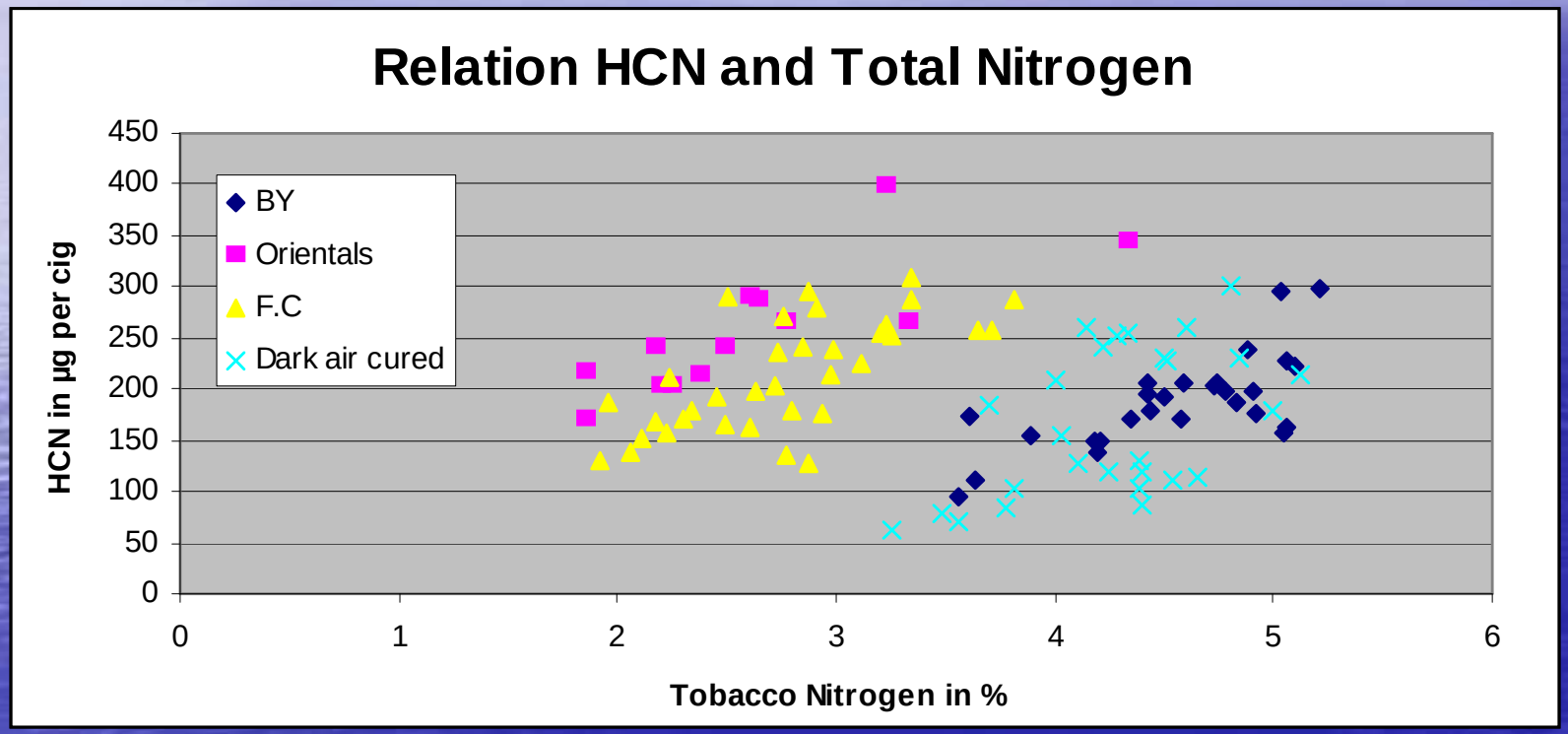
US blend / chemicals report	%
ASN	0.63
GLN	1.5
Estimated proteins	13
Total Nitrogen	3.1

Probable mechanism:

[^^^^CO-NH^^^^] >>> HCN

Modeling:

Relation between HCN amounts in MSS and Total Nitrogen in tobacco as is (expressed in % of DM).

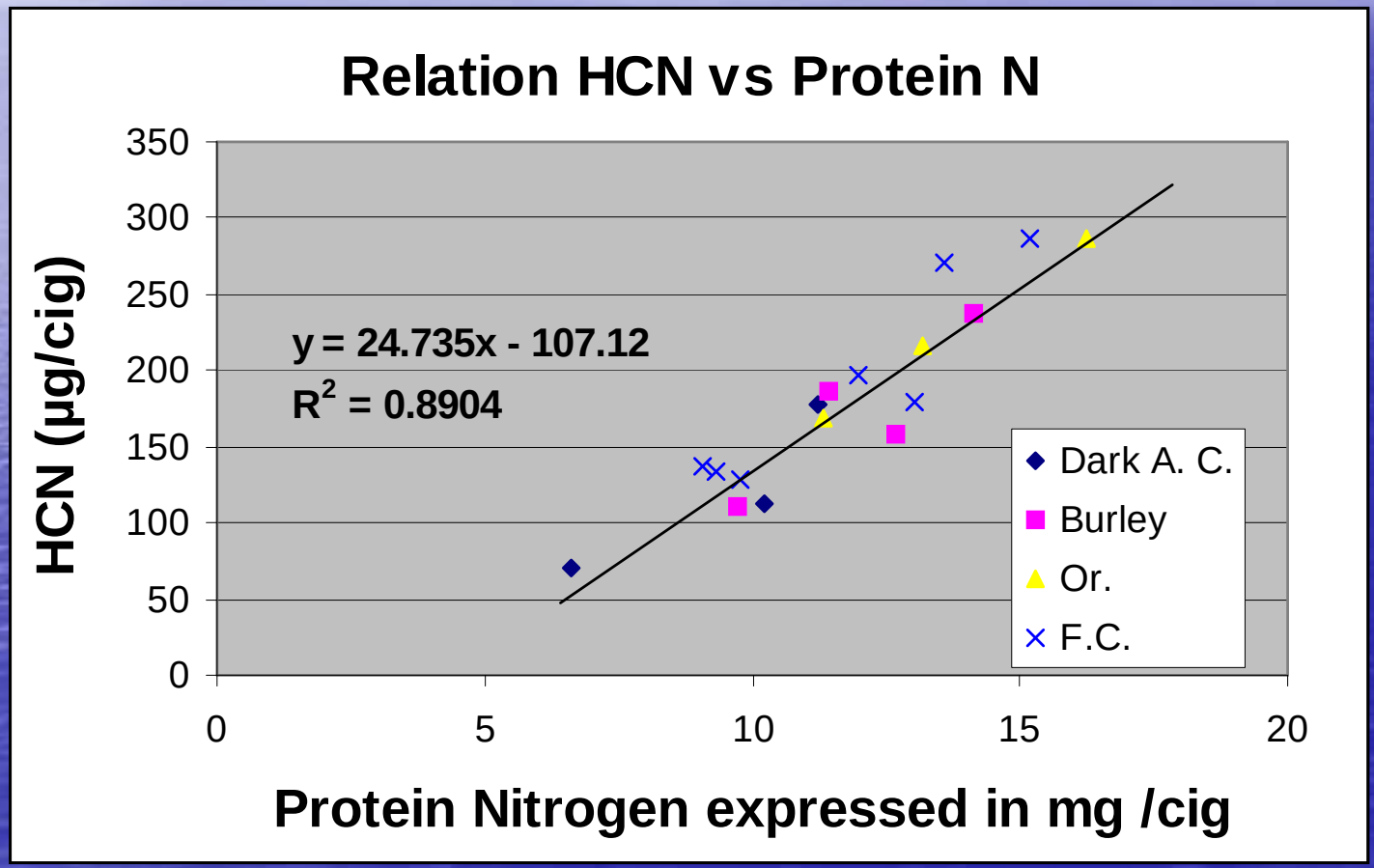


105 representative tobacco lots, including dark air cured.

	Slope	Intercept	R^2
Flue Cured	74.79	7.09	0.46
Oriental	72.11	67.76	0.62
Burley	68.51	-124.13	0.51
Dark air cured	81.92	-182.19	0.27

Modeling (contd.):

Protein Nitrogen = Difference of N amounts in coagulated extract with and without pepsin digestion. (Modified Wegner E. approach).



17 tobacco lots, including all tobacco types.

Conclusions:

- **Spiking approach confirms tobacco peptides or amides compounds contribution on HCN formation in smoke.**
- **Great implication of proteins or polypeptides on HCN response, compared to amides potential in tobacco.**
- **Right method for screening of potential precursors but limited for protein estimation.**
- **No general model without protein determination.**
- **Reliable efficiency of Protein Nitrogen protocol for modeling.**