62nd TSRC Nashville, Tennessee USA.

# NITROGEN COMPOUNDS ON MAINSTREAM SMOKE AND TOBACCO PRECURSORS.

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An IMPERIAL TOBACCO GROUP company

# Objective :

**To evaluate effective contribution of tobacco precursors on nitrogenous compounds delivery in mainstream smoke.**

# Structure:

#1 - Introduction

#2 - Methodology

#3 - Precursors list and experimental validation

#4 - Impact and contribution of precursors

#5 - Conclusions

# Methodology:

- Spiking of potential precursors at different amounts on US blend prior to cigarette making. (NTM and tobacco weight = constant).
- Mainstream smoke analysis with classical methods.
- Smoking tests under ISO conditions.

**Constraints:**

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

# Precursors list and controls:

**Proteins:** Albumin and $\gamma$ Globulin.

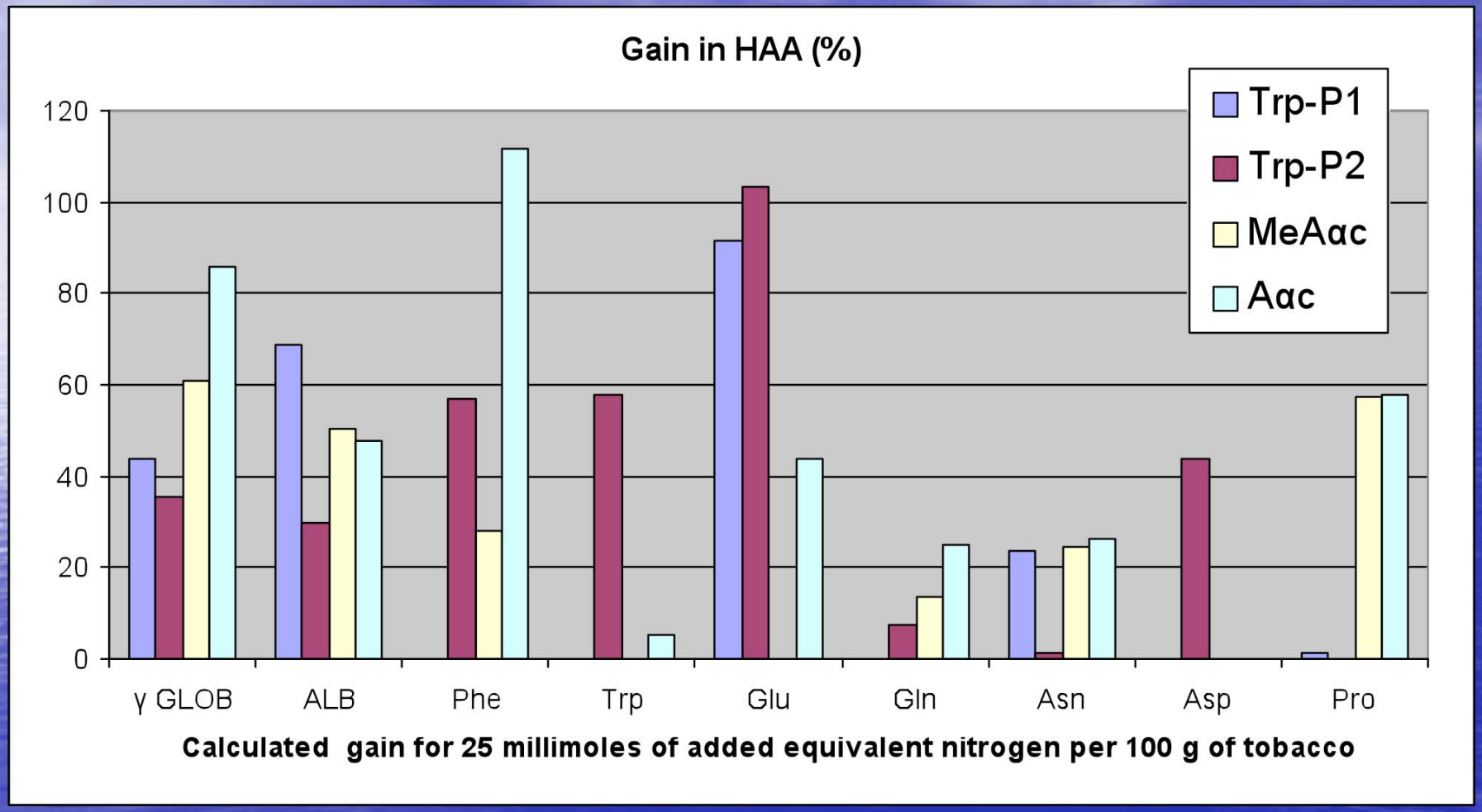
**Amino-acids:** ASN, ASP, PRO, PHE, TRP, GLU and GLN.

Different doses depending on availability and cost.

- Enrichments checked by nitrogen gain or by amino-acid quantification:
  > Accordance between estimated and expected doses.

- ISO smoking tests results on different variants:
  > No significant effect of precursor type or dose on yields.

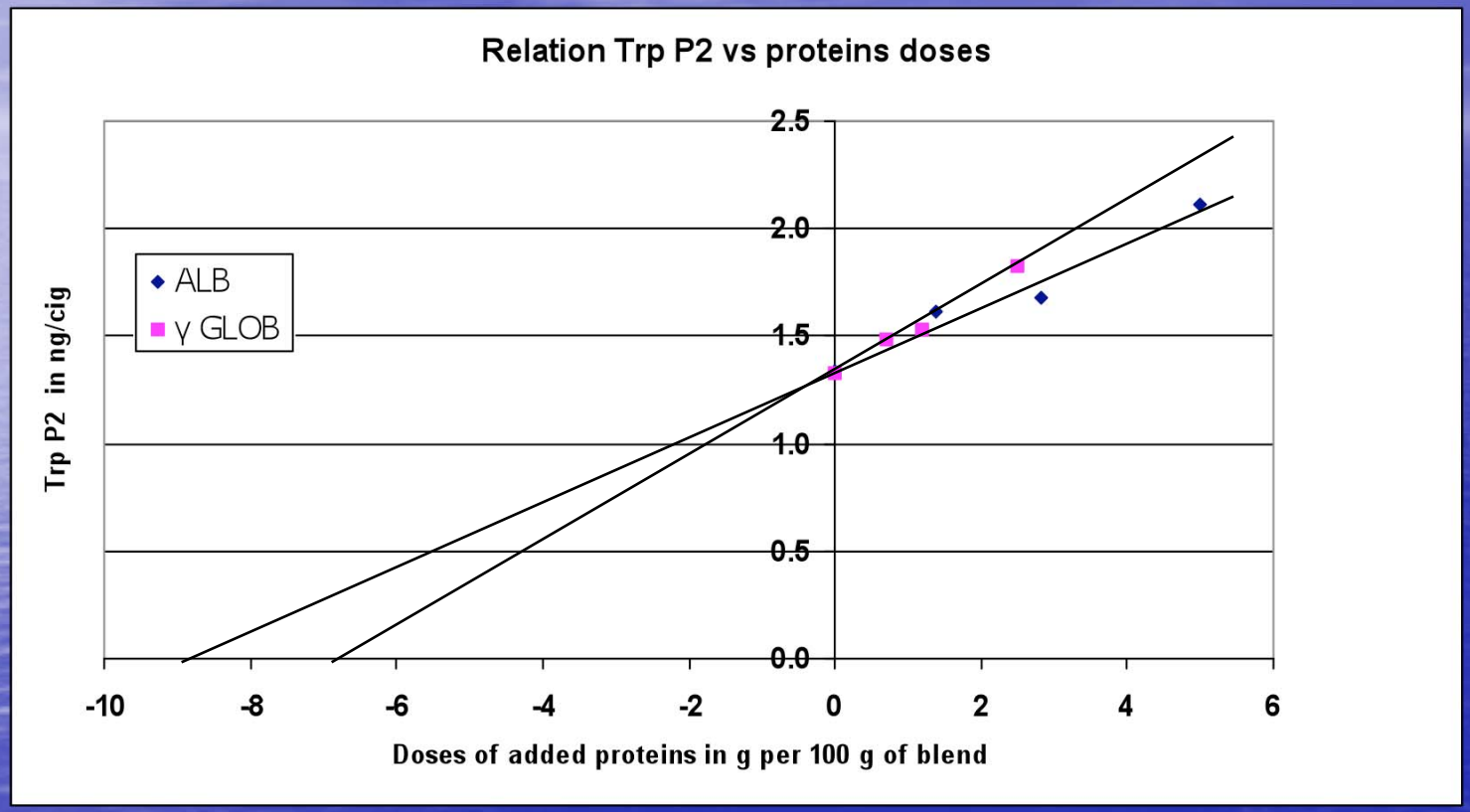
# Tobacco precursors impact on HAA in MSS:




- Great difference of contributions on HAA formation.

# Precursors estimation in a non-enriched blend:

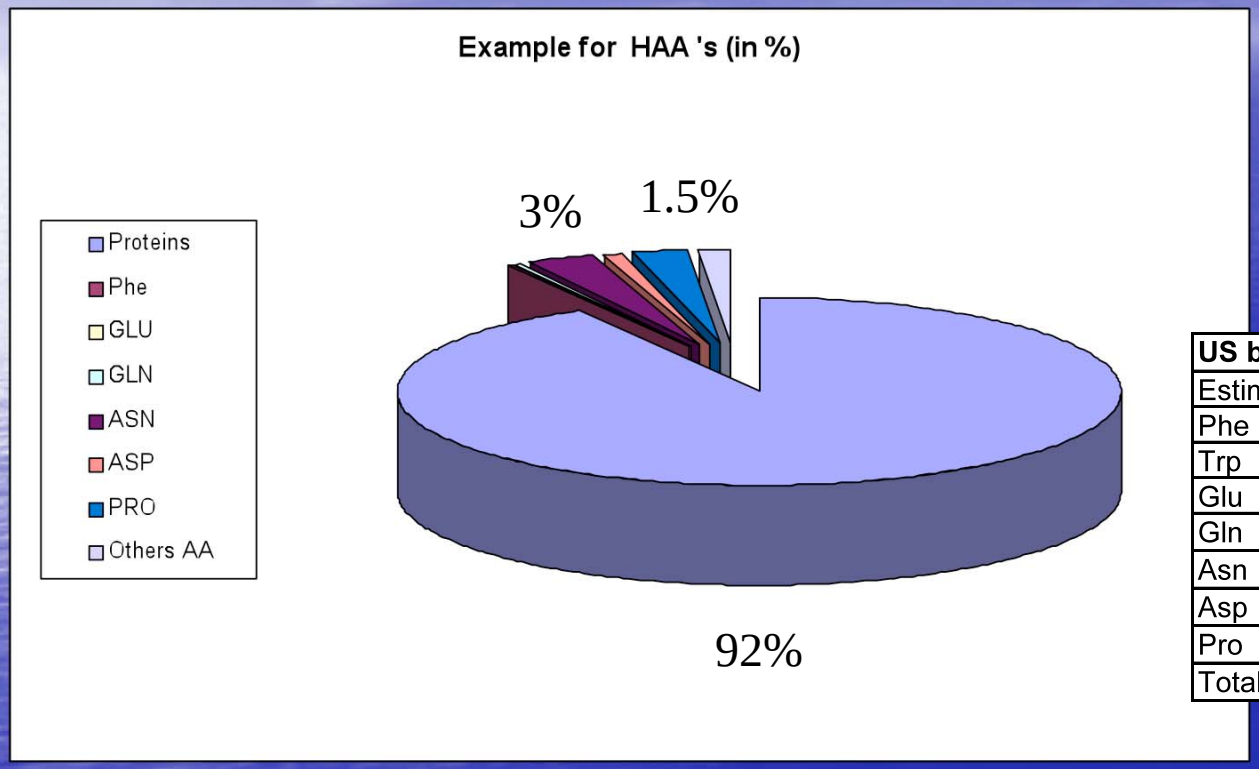
One example with Trp P2




By using spiking approach, possible estimation of proteins amount.
In this blend: Between 7 and 9% of proteins >> Average: 8% (~1.3% expressed in Nitrogen). > Dosed Protein : 8.8% (Protein N = 1.4%).

- Trp P2 amount: Equivalent to ~ 3% of Glu, 4% of Trp or 7% of Phe.

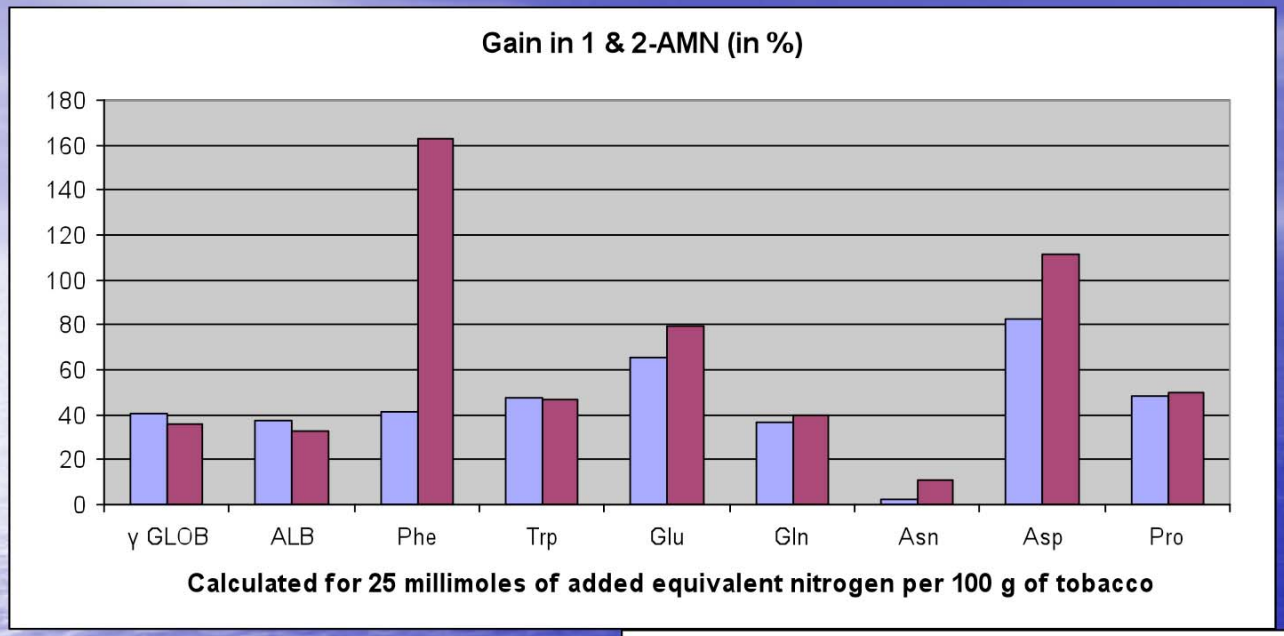
# Relative contribution of precursors for non-enriched blended cigarette:



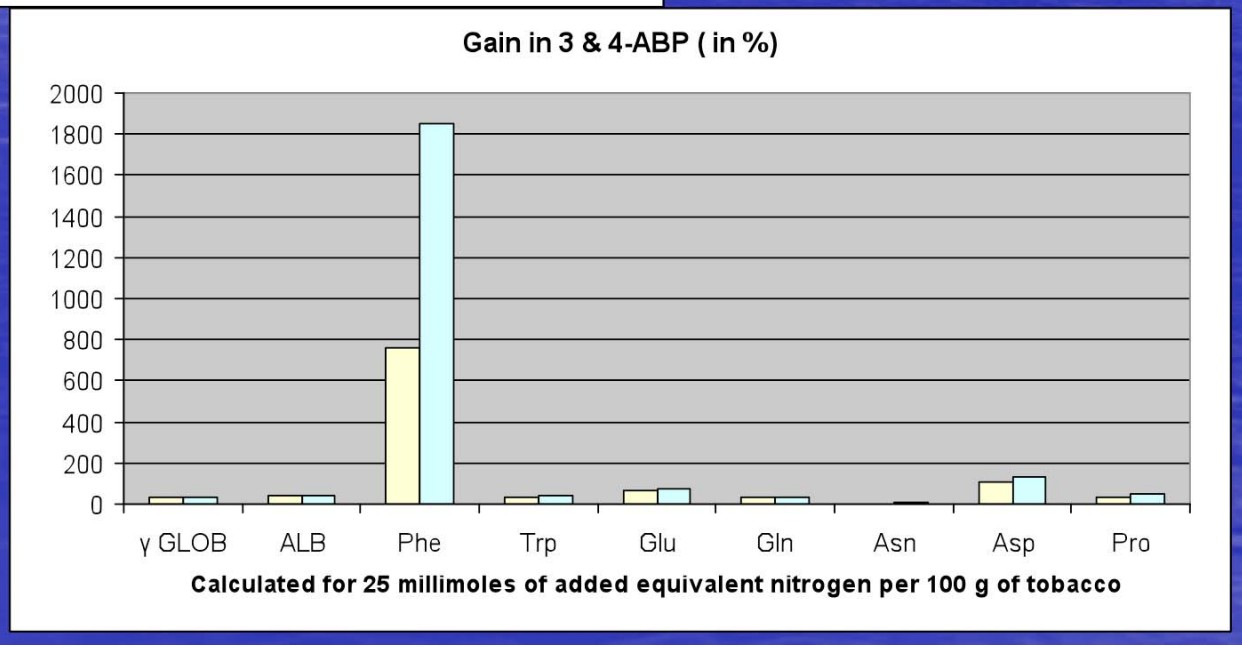

| US blend chemicals report | % or (ppm) |
|---|---|
| Estimated proteins (N prot * 6.24) | 8.8 |
| Phe | (510) |
| Trp | (497) |
| Glu | (624) |
| Gln | (902) |
| Asn | 0.65 |
| Asp | 0.24 |
| Pro | 0.56 |
| Total Nitrogen | 2.71 |

- **Great implication of proteins on HAA deliveries, compared to amino acids content in tobacco or blend.**

# Tobacco precursors effect on PAA in MSS:

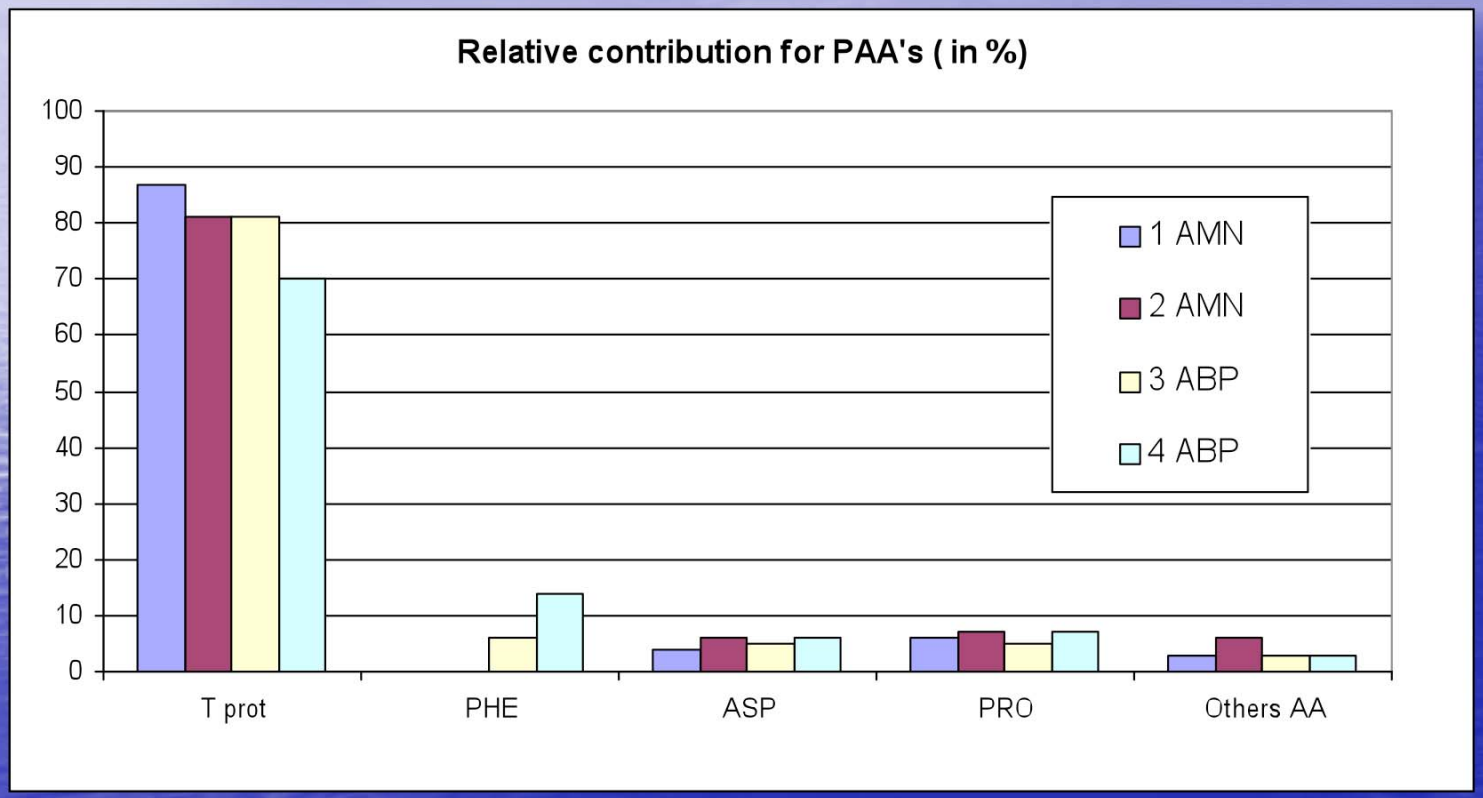



- Numerous precursors involved in 1 & 2 amino naphthalene formation.



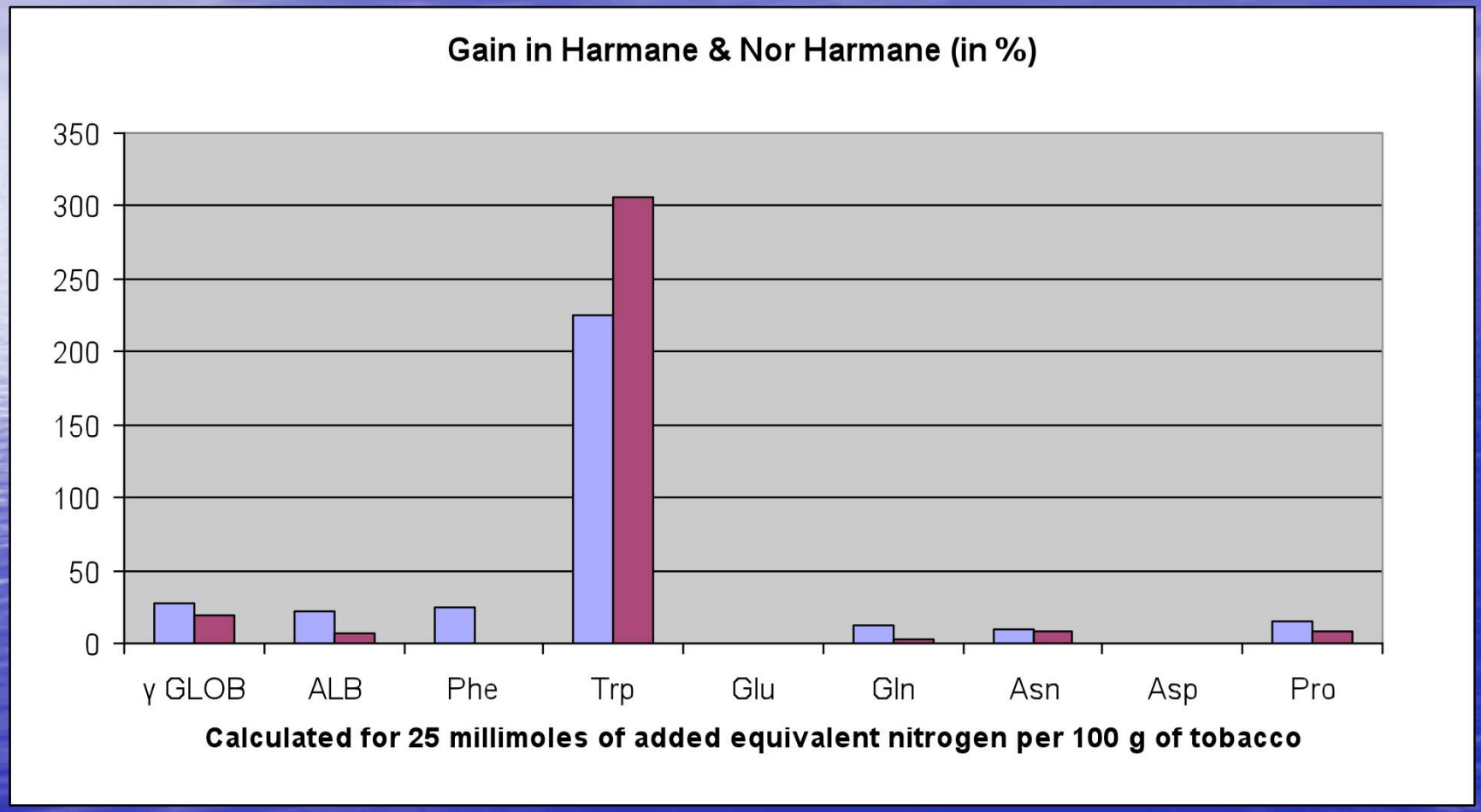

- Strong impact of PHE on 3 & 4 amino biphenyl.

# Relative contribution of precursors for non-enriched blended cigarette:



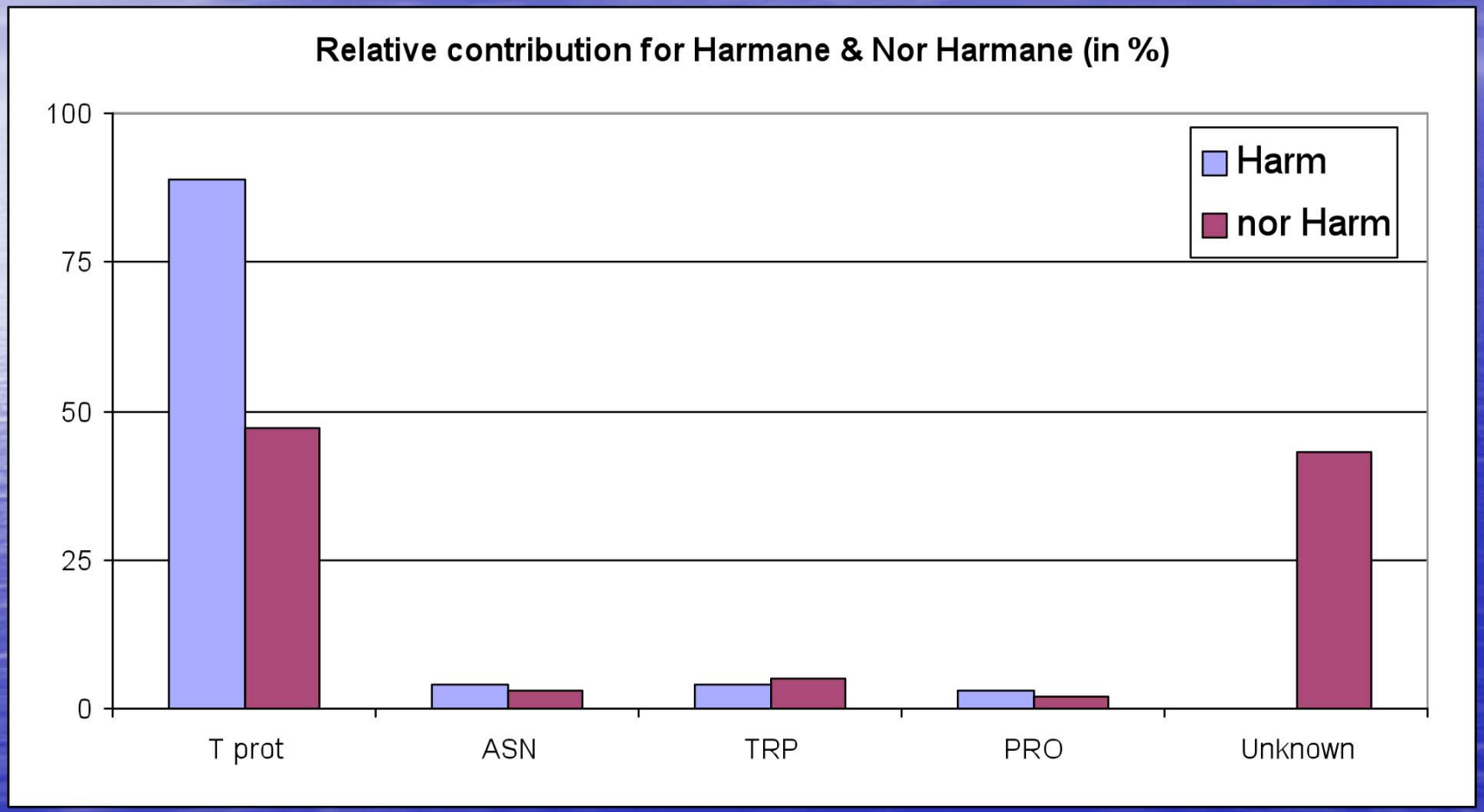

- **Great implication of proteins on PAA delivery, compared to amino acids potential in tobacco or blend.**

# Tobacco precursors effect on Harmane & Nor Harmane in MSS:



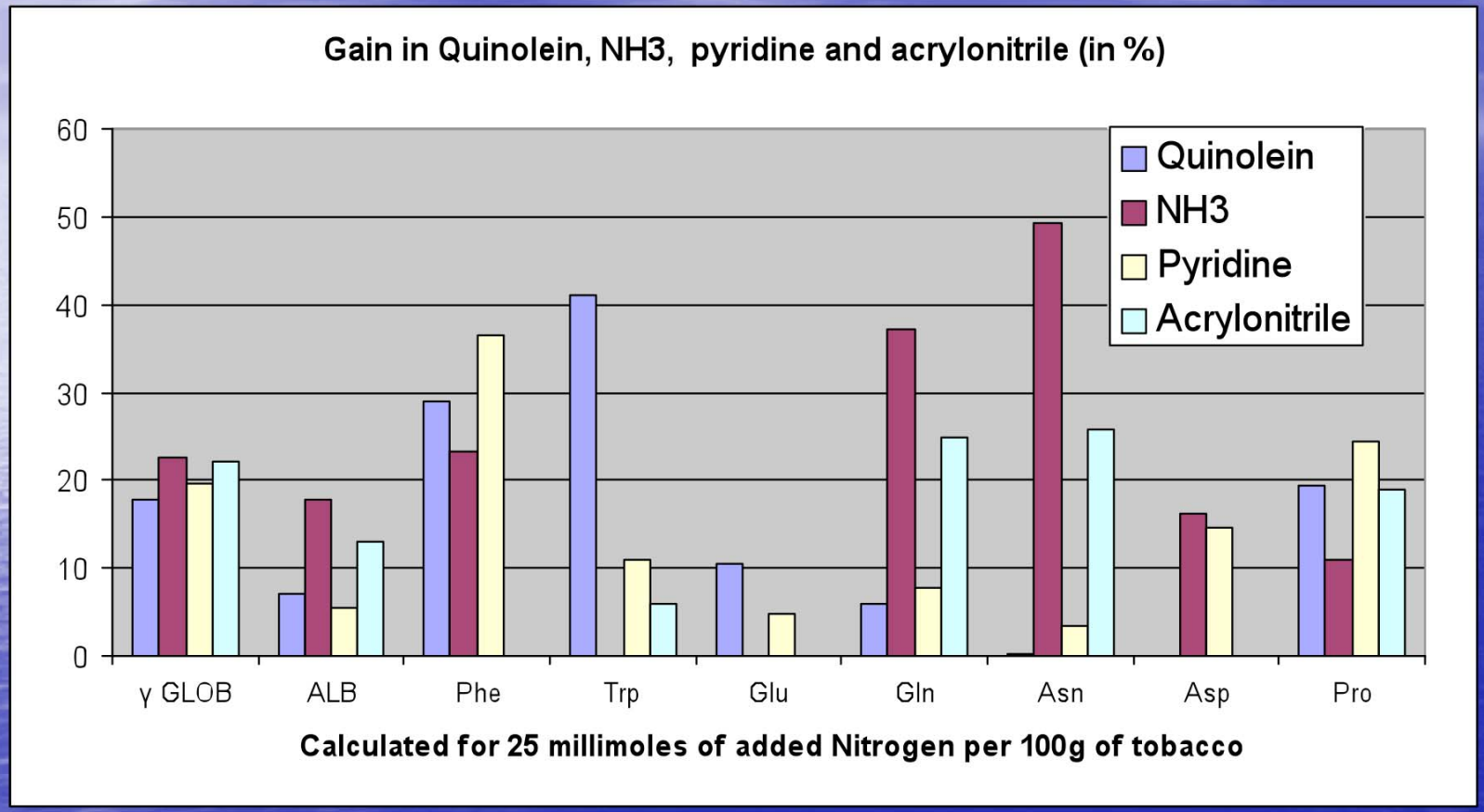

- Strong impact of Tryptophane on $\beta$ Carbolines formation.

# Relative contribution of precursors for non-enriched blended cigarette:




- **Great implication of tobacco proteins on β Carbolines.**

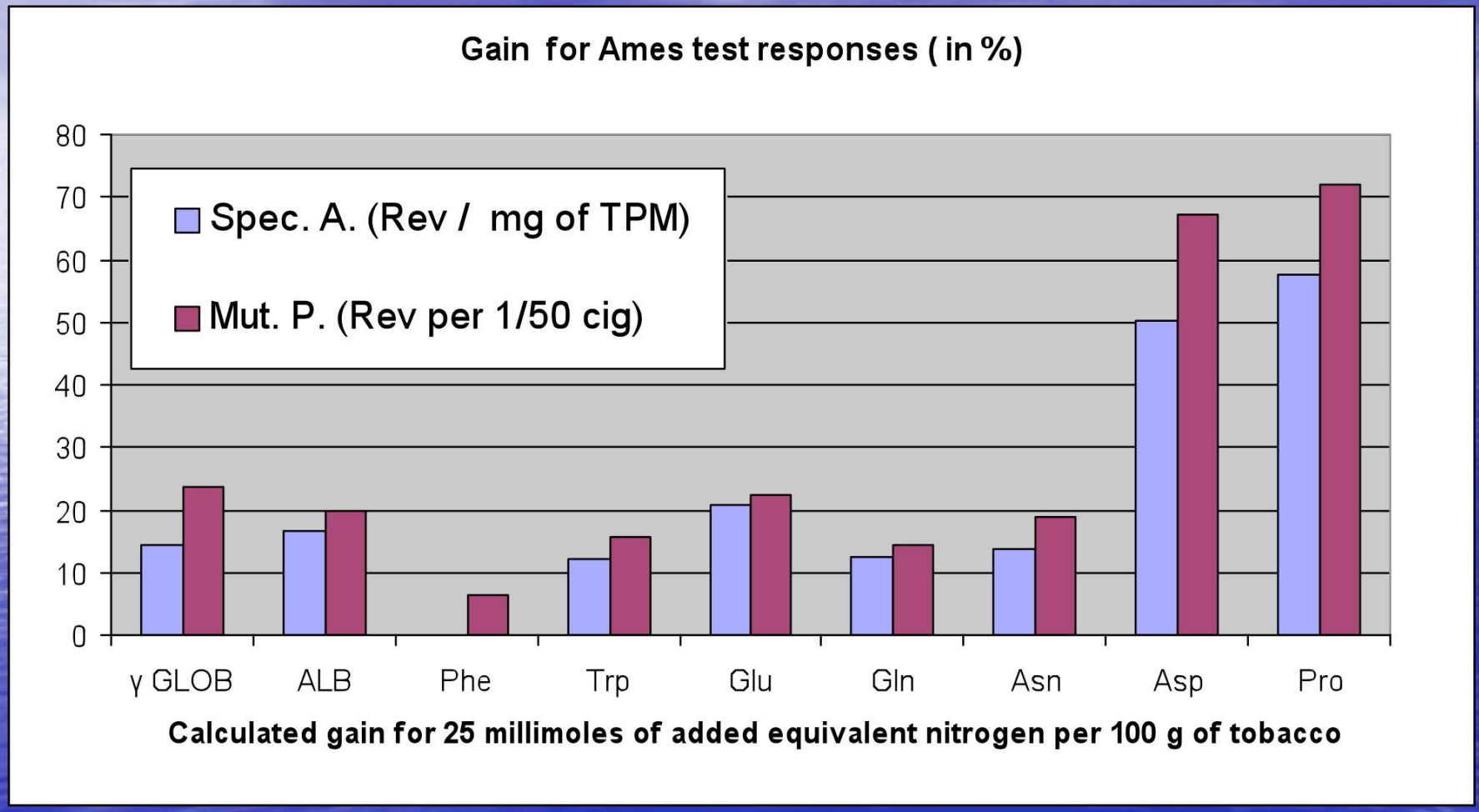
# Precursors impact on other nitrogenous compounds:




- **Significant impact of Tryptophane and Phenylalanine on quinolein formation.**
- **Great effect of amides on NH3 in smoke (GLN & ASN).**
- **Numerous precursors involved in pyridine and acrylonitrile delivery.**

# Precursors impact on Ames test responses:

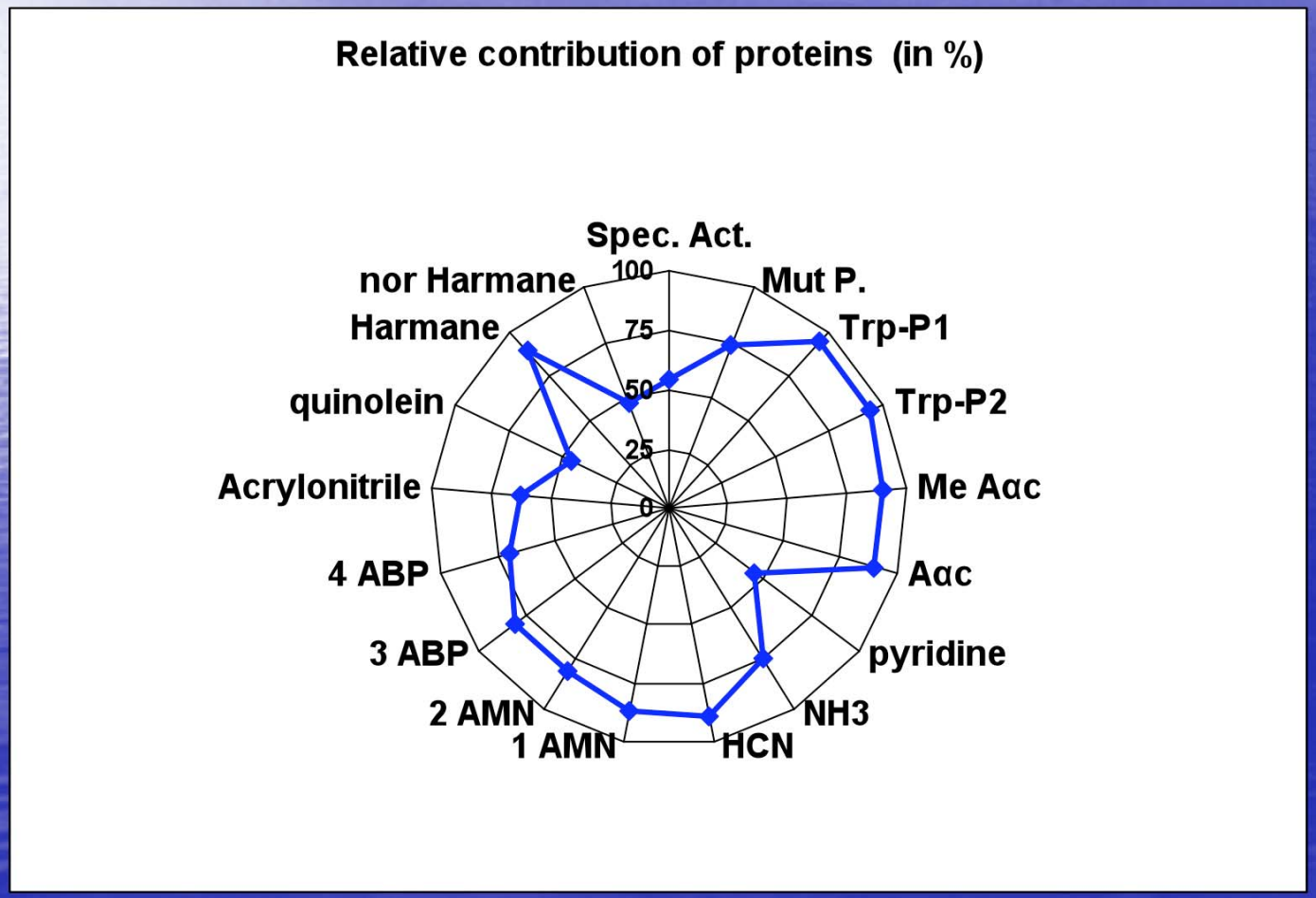
(Strain TA 98 with activator S9)




- **Significant effect of ASP, PRO and proteins on Ames test responses.**

# Implication level of proteins on MSS chemicals and Ames test responses:

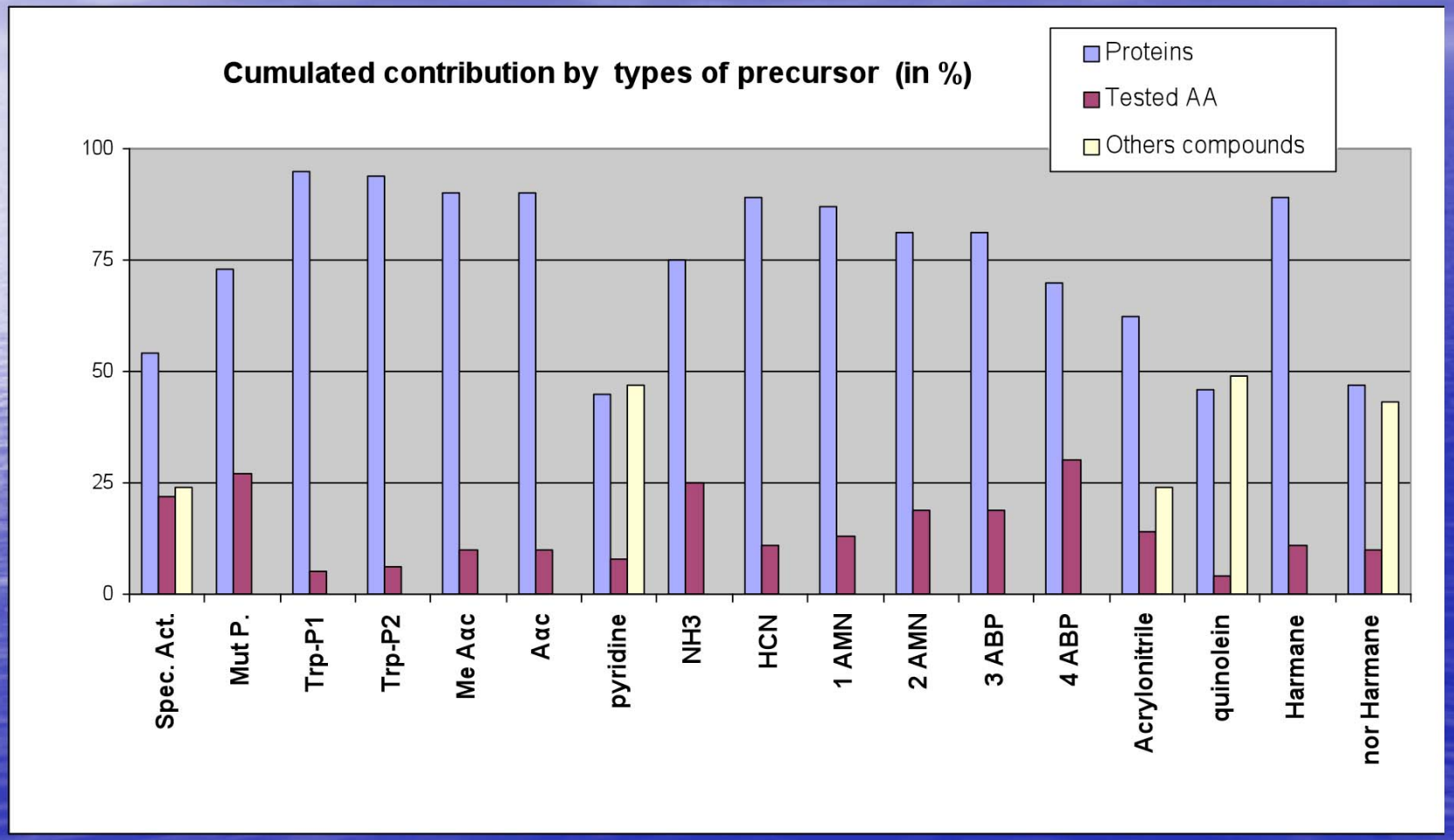
Estimated from a non-enriched blend cigarette.




Large implication of proteins on nitrogenous compounds and Ames responses in smoke (> 45 %).

# Contribution of precursors on MSS chemicals and Ames tests responses:

(Calculated for a non-enriched blend cigarette).




- Several smoke compounds are completely explained by tested precursors.

# Conclusions:

- **Appropriate method for screening of potential precursors.**
- **Spiking approach confirms proteins and amino acids involvement in nitrogenous compounds in smoke.**
- **Great implication of proteins on these responses, compared to amino acids potential in tobacco.**
- **No obvious relationship between MSS nitrogenous compounds and Ames test responses.**