PMI RESEARCH & DEVELOPMENT

In vitro comparison of combustible vs. non-combustible tobacco products

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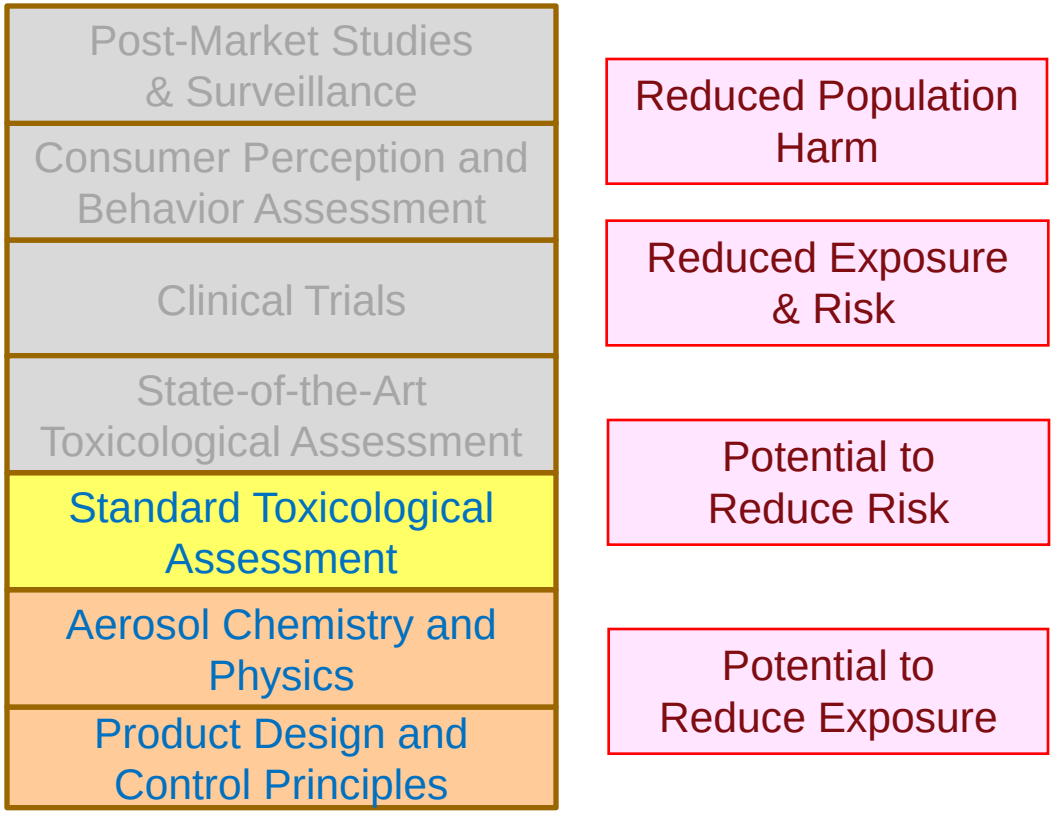
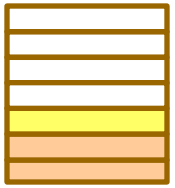
PMI's assessment strategy



Non-clinical assessment



Product design, manufacturing and controls

- pMRTP* production is under control, meets required quality standards and consistently delivers according to specifications. It is then ready to enter the formal product assessment phase

Non-clinical evidence:

- Chemical analysis – for known and novel aerosol constituents
- Toxicological assessment - in cell systems and laboratory animals

To do what?

- Determine if the product is acceptable for:
  - Use by internal/external volunteer panels in support of product development
  - Clinical studies
  - Market Launch

*pMRTP ; prototype Modified Risk Tobacco Product

Sample preparation

Standard Toxicological Assessment

- Conventional cigarette (CC) smoke or pMRTP aerosol is generated using a smoking machine operating to a standard smoking regime
- The total particulate matter (TPM) is collected on a glass fiber filter (Cambridge filter) and extracted with DMSO for its application in NRU, Ames and MLA assay
- The gas/vapor phase (GVP) passing through the Cambridge filter is collected by bubbling through an impinger containing ice-cold phosphate-buffered saline

In vitro assessment

Standard Toxicological Assessment

- Cytotoxicity
  - Neutral Red Uptake (NRU) assay according to the INVITTOX protocol No. 3a. Using mouse embryo BALB/c 3T3 cells
- Bacterial cell mutagenicity
  - Five strains of *S. typhimurium:* TA98; TA1537;TA100; TA1535; TA102 (in the presence and absence of rat S9 metabolic activation system according to OECD guideline 471
- Mammalian cell mutagenicity
  - *In vitro* Mouse Lymphoma Assay (MLA) performed using L5178Y tk+/- cells according to OECD guideline 476

Objectives of the assessment

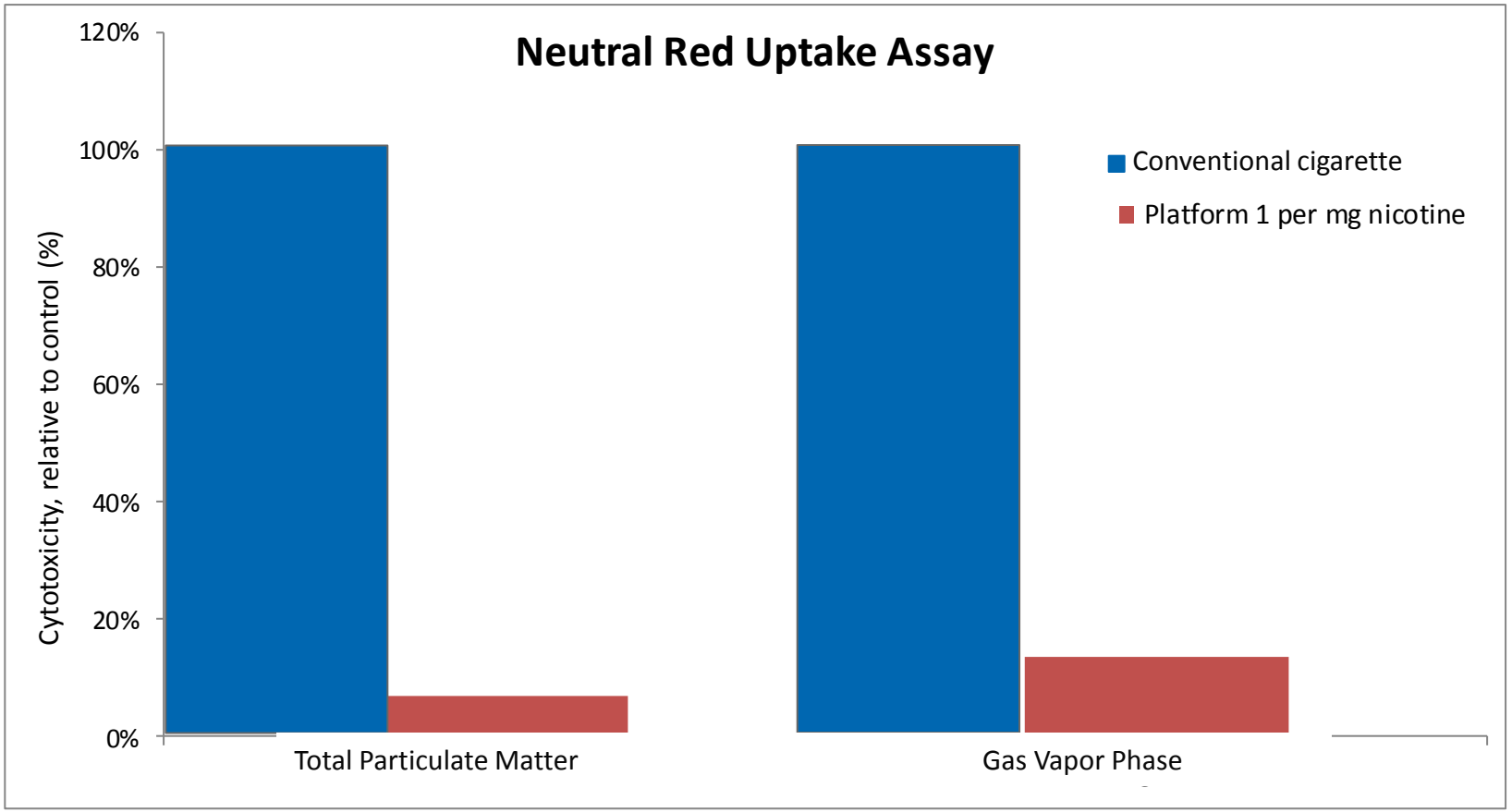
Standard Toxicological Assessment

- To assess whether ‘heating instead of burning’ tobacco produces a much less complex aerosol resulting in a substantially reduced *in vitro* hazard potential
- To provide data for hazard characterization in a tiered assessment strategy
- To assess the pMRTP prior to its use in human studies in adult smokers
  - No increased or new hazard when compared to CC

In Vitro Data for a pMRTP

Standard Toxicological Assessment

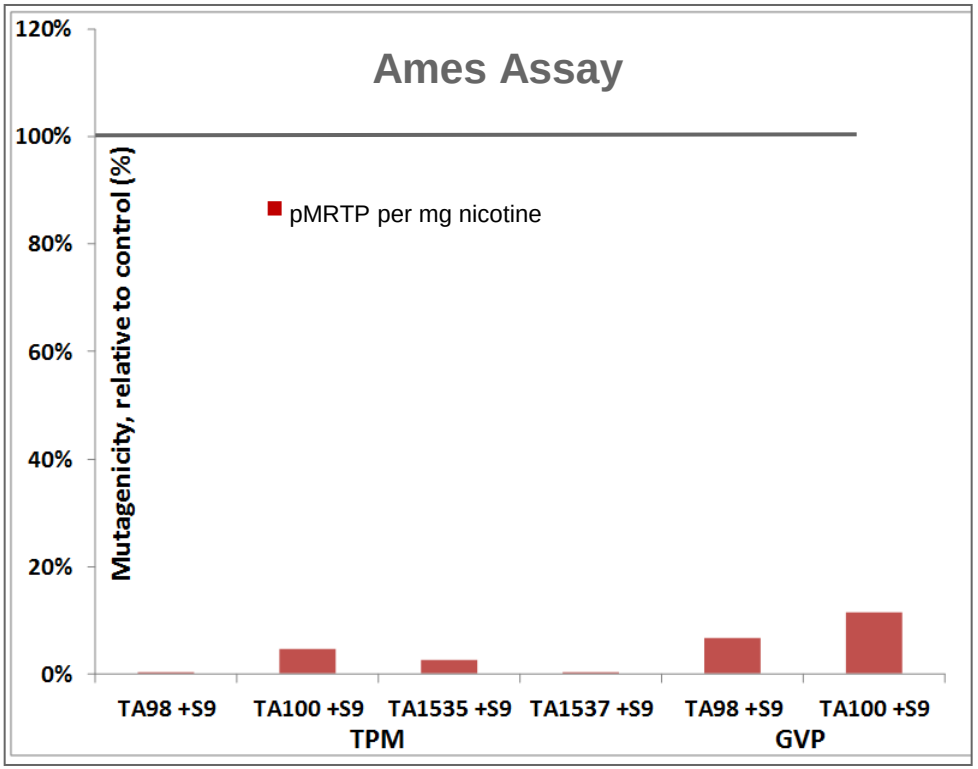
Cell death substantially decreased compared to reference cigarette



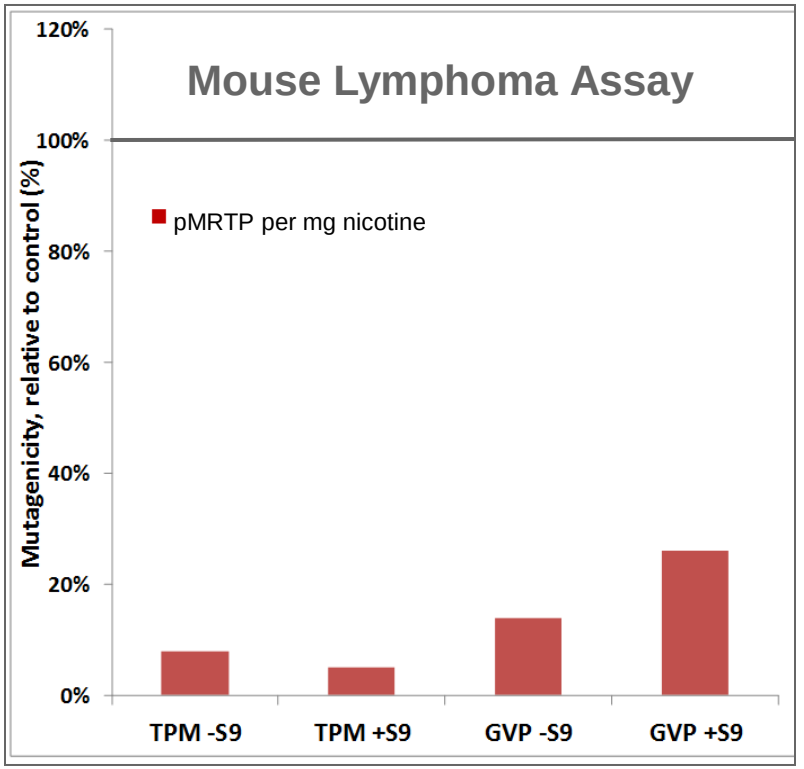
In Vitro Data pMRTP

Standard Toxicological Assessment

Cell mutation substantially decreased compared to conventional cigarette



pMRTP and the reference cigarette 3R4F.



pMRTP and the reference cigarette 3R4F.

Summary and conclusions

Standard Toxicological Assessment

- The reduced yield of HPHCs from the pMRTP compared to CC translates to:
  - Reduced cytotoxicity
  - Reduced mutagenicity in a bacterial cell system
  - Reduced mutagenicity in a mammalian cell system
- Results confirm reduced hazard potential of pMRTP compared to CC
- No indication of new cytotoxic or genotoxic hazards being introduced into the pMRTP
- Results cannot be used to measure human risk but form part of the evidence package in support of the overall assessment strategy