**Discrimination of Cigarette Mainstream Smoke Condensates with the Salmonella Reverse Mutation Assay**

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**Introduction**

The Salmonella reverse mutation assay is used in the evaluation of cigarette smoke condensates (CSC) for potential mutagenic activity. The reproducibility of this assay, and its power to discriminate between the mainstream smoke condensates (MSC) of various research cigarettes is described below. The assay standardization is based on a study design with a total of 66 research cigarette types or an equivalent number of condensates prepared for each cigarette type. This study reports a blinded evaluation of 12 research cigarette types. The Salmonella reverse mutation assay was applied to these CSC and compared to a standard reference cigarette (1R4F) by statistical means.

**Materials and Methods**

- **Cigarettes**:
  - 57 different research cigarettes
  - American-blended single blends
  - 6 cigarettes made from single blends of American-blended mixtures
  - 57 different research cigarettes
  - Metabolic promutagen activation by S9 of livers from rats treated with 2-AAF

- **Mainstream Smoke Condensate**
  - Smoke condensates were collected in glass impaction traps
  - Condensate dissolved in dimethyl sulfoxide
  - 4 condensate batches per cigarette type, each prepared, assayed, and stored according to ISO standards (EHC-CA)

- **Internal Controls**
  - MSC was collected in glass impaction traps
  - Condensate dissolved in dimethyl sulfoxide
  - Internal controls were added to each plate

- **Mutagenicity Assay**
  - A modified TA98 and TA100 assay for the evaluation of cigarette smoke condensates (CSC) was used
  - Condensate dissolved in dimethyl sulfoxide
  - 4 condensate batches per cigarette type, each prepared, assayed, and stored according to ISO standards (EHC-CA)

**Results**

- **Dosage Response Curve for 1R4F-MSC**
  - Dose-response curves were determined for each cigarette type
  - Regression analysis with Poisson-weighted data
  - Results given as mean specific mutagenicity per cigarette (revertants/cig.)

- **Statistical Analysis**
  - Regression analysis with Poisson-weighted data
  - Results given as mean specific mutagenicity per cigarette (revertants/cig.)
  - One-way analysis of variance for each composition followed by Fisher’s least significant difference (LSD) test for pair-wise comparison

- **Reproducibility**
  - Specific mutagenicity Relative to 1R4F
  - Mutagenicity per Cigarette

**Discussion**

- **Influence of Reconstituted Leaf Process**
  - Influence of reconstituted leaf process on specific mutagenicity

**Conclusion**

- **Optimization of the Salmonella reverse mutation assay**
  - Optimization of the Salmonella reverse mutation assay resulted in an increased reproducibility of the assay
  - The observed range of specific mutagenicity relative to 1R4F was similar to the literature

**References**


**Author Comment**