

# Preclinical Testing of Flavors in E-vapor Products Part 4: Flavor Transfer from the Liquid to the Aerosol for Inhalation Exposure

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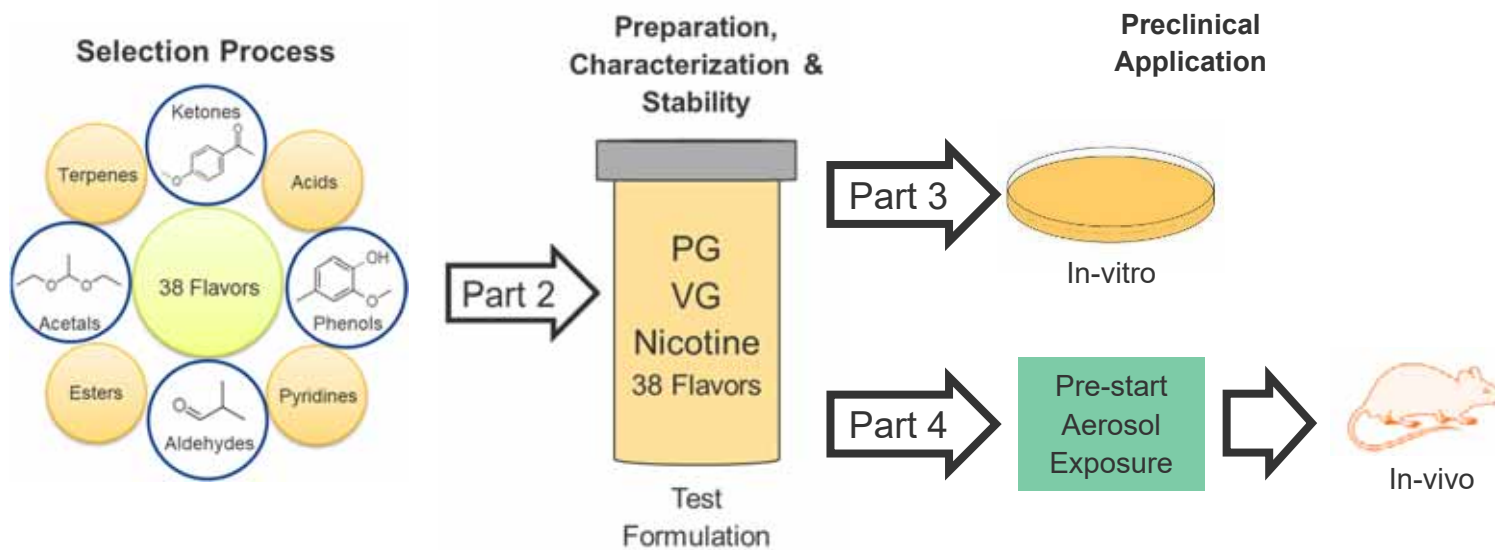


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# Overview

- Preclinical testing of flavors in e-vapor products, Part 1: selection of representative flavor mixtures for toxicological evaluations using a structural grouping approach
- Preclinical testing of flavors in e-vapor products, Part 2: preparation and stability characterization of representative flavor mixtures
- Preclinical testing of flavors in e-vapor products, Part 3: in vitro cytotoxicity and genotoxicity of representative flavor mixtures
- Preclinical testing of flavors in e-vapor products, Part 4: flavor transfer from the liquid to the aerosol for inhalation exposure

# Overview



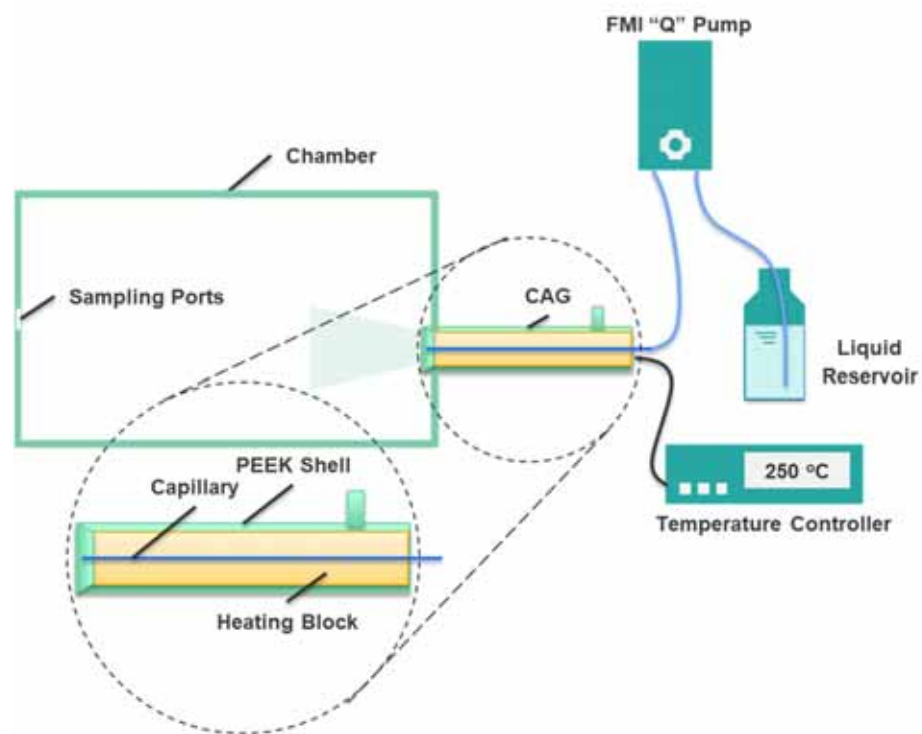
PG: propylene glycol  
VG: vegetable glycerol

# Objective

- To generate aerosols using designated test formulations
- To characterize the generated aerosols
- To confirm the flavor transfer from formulation to aerosols

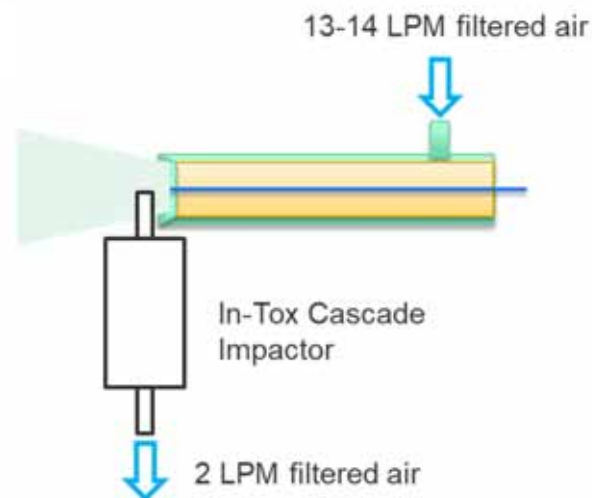
# Aerosol Generation

- Test Formulation with and without nicotine



# Aerosol Size Distribution

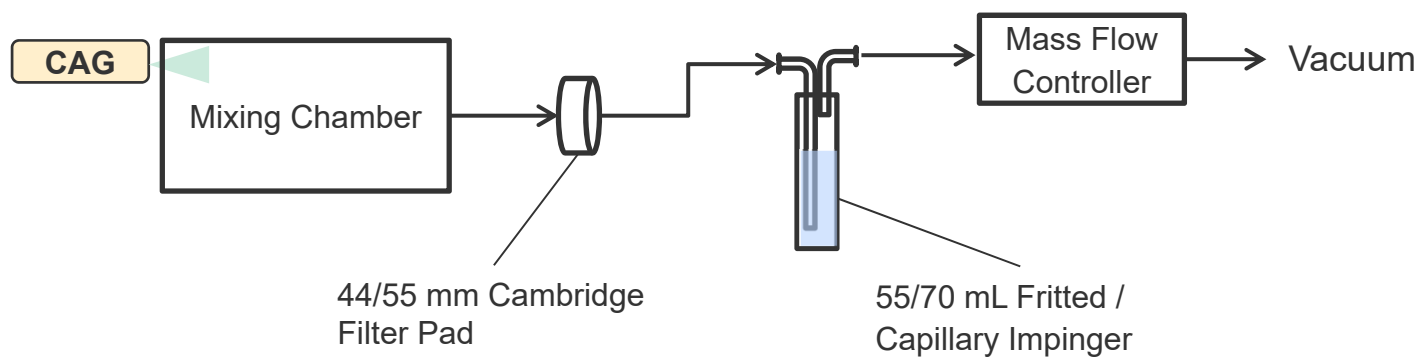
- Method
  - Cascade impactor



|                        | Test Formulation w/<br>Nicotine<br>(n = 4) | Test Formulation w/o<br>Nicotine<br>(n = 4) |
|------------------------|--|---|
| MMAD ( $\mu\text{m}$ ) | $0.97 \pm 0.07$                            | $1.23 \pm 0.06$                             |
| GSD                    | $1.77 \pm 0.18$                            | $1.82 \pm 0.13$                             |

# Aerosol Sampling for Chemical Analysis

| Analyte of Interest    | Cambridge Filter Pad | Impinger                     |
|------------------------|----------------------|------------------------------|
| Aerosol Mass           | 44 mm                | No                           |
| Nicotine, PG, Glycerin | 44 mm                | No                           |
| Selected Carbonyls     | 44 mm                | DNPH derivatization solution |
| Selected Flavors       | 55 mm                | Ethanol (-70°C)              |
| pH                     | No                   | Fritted; KCl solution        |



# Analytical Characterization

| Analyte           | Test Formulation w/ Nicotine (N = 3) |                         |                       | Test Formulation w/o Nicotine (N = 3) |            |                       |
|-------------------|--------------------------------------|-------------------------|-----------------------|---------------------------------------|------------|-----------------------|
|                   | Liquid                               | Aerosol                 | Transfer <sup>b</sup> | Liquid                                | Aerosol    | Transfer <sup>b</sup> |
| Aerosol Mass (mg) | NA                                   | 98.1±2.0                | NA                    | NA                                    | 108.2±1.8  | NA                    |
| Ethanol (mg/g)    | 20.44±0.13                           | BLOQ                    | NA                    | 20.19±0.23                            | BLOQ       | NA                    |
| Glycerol (mg/g)   | 144.3±0.3                            | 146.2±2.1 <sup>a</sup>  | 101%                  | 146.1±0.5                             | 147.1±3.1  | 101%                  |
| Nicotine (mg/g)   | 20.21±0.17                           | 20.61±0.25 <sup>a</sup> | 102%                  | ND                                    | ND         | NA                    |
| PG (mg/g)         | 580.6±2.14                           | 611.2±14.2 <sup>a</sup> | 105%                  | 625.3±0.99                            | 656.3±26.5 | 105%                  |
| Water (mg/g)      | 63.11±0.89                           | 79.90±2.37 <sup>a</sup> | 127% <sup>c</sup>     | 55.81±0.71                            | 73.81±0.71 | 132% <sup>c</sup>     |

<sup>a</sup>. The values were normalized by the collected aerosol mass.

<sup>b</sup>. The transfer was calculated as  $\text{Transfer (\%)} = \frac{\text{Concentration in Aerosol } (\frac{\text{mg}}{\text{g}})}{\text{Concentration in E-liquid } (\frac{\text{mg}}{\text{g}})} \times 100\%$ .

<sup>c</sup>. Water exceeded 100% by a wide margin due to the hygroscopicity of PG and Glycerin.

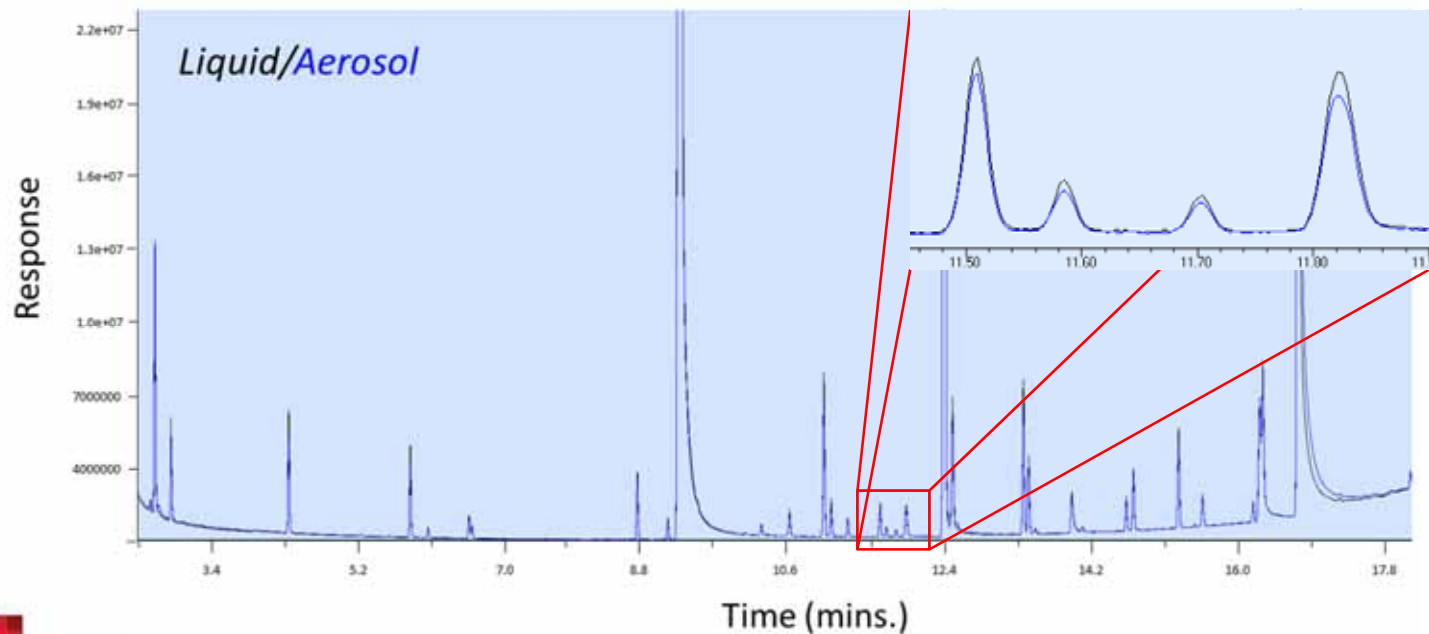
NA = not applied; ND = not detected; BLOQ = below the limit of quantification.





# Flavor Transfer

- Test formulations contained 38 flavors.
- 22 out of 38 (Lab 1) were analyzed in both the liquid and the aerosol.



## Selected Carbonyls in the Aerosol

|                                    | Blank<br>(n = 3) | Carrier<br>(PG/VG/Nicotine/<br>Water) (n = 3) | High w/ Nicotine<br>(n = 3)   | High w/o Nicotine<br>(n = 3)  |
|------------------------------------|------------------|---|-------------------------------|-------------------------------|
| Aerosol Mass (mg)                  | 100 mg           | 107.2 ± 5.4                                   | 106.7 ± 1.3                   | 116.1 ± 1.5                   |
| Formaldehyde (µg/g) <sup>c</sup>   | < LOQ            | 8.71 ± 0.57                                   | 4.98 ± 0.15                   | 5.88 ± 0.24                   |
| Acetaldehyde (µg/g) <sup>c</sup>   | 3.09 ± 0.11      | 8.34 ± 0.89                                   | <b>Above 1000<sup>b</sup></b> | <b>Above 1000<sup>b</sup></b> |
| Acrolein (µg/g) <sup>c</sup>       | < LOD            | 1.63 ± 0.20                                   | 5.36 ± 0.65                   | 2.37 ± 0.13                   |
| Crotonaldehyde (µg/g) <sup>c</sup> | < LOD            | < LOD   | 10.57 ± 0.75                  | 8.18 ± 0.17                   |

<sup>a</sup>. Assumes 100 mg for calculation purposes;

<sup>b</sup>. Approximations - Above Calibration Curve;

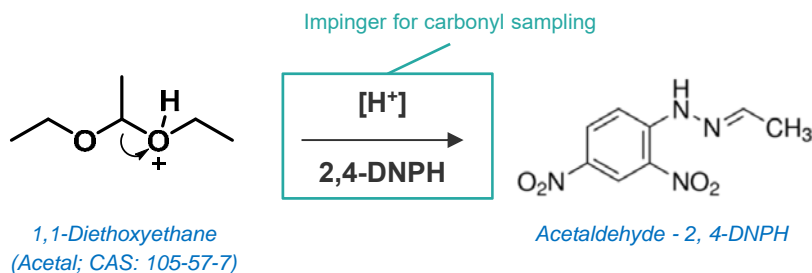
<sup>c</sup>. Reported values were normalized to the collected aerosol mass.



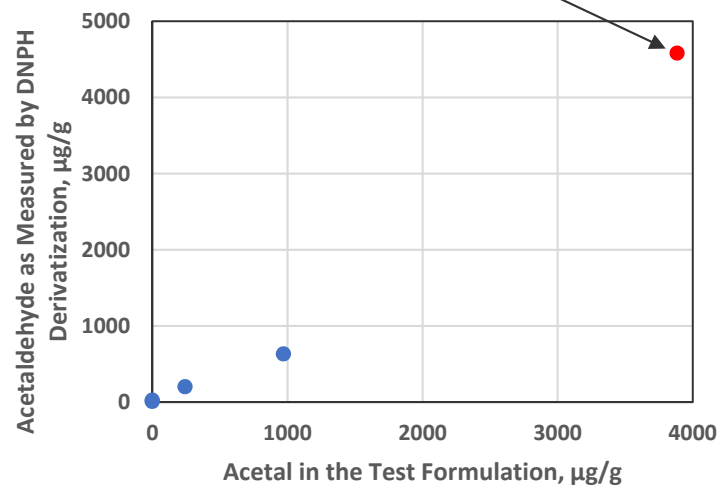
*Where did acetaldehyde come from?*

# 1,1-Diethoxyethane in Pre-blend Ic Detected as Acetaldehyde

- 1,1-diethoxyethane detected as acetaldehyde in the carbonyl analysis due to the sampling limitation
- 1,1-diethoxyethane as a flavor was transferred to the aerosol around 100% by GC-MS method (Lab 2).



Approximation (above calibration curve)



1,1-Diethoxyethane (acetaldehyde diethyl acetal)

# pH of the Aerosol

|                    | Test Formulation w/o Nic | Test Formulation w/ Nic |
|--------------------|--------------------------|-------------------------|
| Liquid pH (n = 3)  | 4.6                      | 7.7                     |
| Aerosol pH (n = 3) | 4.7                      | 7.6                     |



*pH adjustment in the formulation?*

|                   | Test Formulation w/o Nic<br>(pH adj. w/ NaOH) |
|-------------------|---|
| Liquid pH (n = 1) | 7.1   |
| Aerosol pH (n=3)  | 4.6   |



After <1 hr



**Always characterize the test atmosphere for confirmation.**

## Summary

- ✓ Flavor transfer from liquid formulation into the aerosol was confirmed.
- ✓ Particle size for both formulations (high with and without nicotine) tested were in the desired range (MMAD<1.6  $\mu\text{m}$ , GSD<2).
- ✓ Nicotine, PG and glycerol matched in formulations and CAG aerosol for the test formulations.
- ✓ Selected carbonyls measured in CAG generated aerosols were consistent with previous studies.
- ✓ Aerosol generated from final formulations had a measured pH between 4 and 8.

# Acknowledgements

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