

**Part Three: Biomarker Changes in Cigarette Smokers Switched to Vuse Solo or Abstinence: A randomized, Controlled Study**

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**Supplementary Table S1. Subject demographics and baseline characteristics**

| Characteristic                         | Vuse Solo (n = 35) | Abstinence (n = 16) |
|----------------------------------------|--------------------|---------------------|
| Age, years                             | 41.2±11.2          | 39.9±8.0            |
| Weight, kg                             | 81.2±16.3          | 83.2±19.4           |
| Height, cm                             | 172.5±8.0          | 172.2±10.4          |
| BMI, kg/m <sup>2</sup>                 | 27.4±6.0           | 28.0±5.4            |
| Sex                                    |                    |                     |
| Male                                   | 20 (57.1%)         | 9 (56.3%)           |
| Female                                 | 15 (42.9%)         | 7 (43.8%)           |
| Ethnicity                              |                    |                     |
| Hispanic/Latino                        | 2 (5.7%)           | 0                   |
| Not Hispanic/Latino                    | 32 (91.4%)         | 16 (100.0%)         |
| Not reported                           | 1 (2.9%)           | 0                   |
| Race                                   |                    |                     |
| White                                  | 26 (74.3%)         | 11 (68.8%)          |
| Black/African American                 | 7 (20.0%)          | 4 (25.0%)           |
| Native Hawaiian/Other Pacific Islander | 0                  | 0                   |
| Asian                                  | 0                  | 0                   |
| American Indian/Alaska Native          | 1 (2.9%)           | 0                   |
| Multiple                               | 0                  | 1 (6.3%)            |
| Not Reported                           | 1 (2.9%)           | 0                   |
| Smoking                                |                    |                     |
| Number of years smoked                 | 27.3±10.9          | 24.4±11.2           |
| Number of cigarettes smoked per day    | 18.3±6.9           | 18.3±7.1            |
| FTND                                   | 6.1±1.6            | 6.0±1.3             |

Data are mean±SD or number (%).

Abbreviations: BMI = body-mass index; FTND = Fagerström Test for Nicotine Dependence

**Supplementary Table S2. Changes in biomarkers of exposure**

|                                              |                                              | Vuse Solo<br>(n=35) |                    |          |         | Abstinence<br>(n=16) |                    |          |         |
|----------------------------------------------|----------------------------------------------|---------------------|--------------------|----------|---------|----------------------|--------------------|----------|---------|
| Urinary Biomarker (units)                    | Tobacco Constituent                          | Day -1<br>(Mean±SD) | Day 5<br>(Mean±SD) | % change | p-value | Day -1<br>(Mean±SD)  | Day 5<br>(Mean±SD) | % change | p-value |
| <b>Aromatic Amines</b>                       |                                              |                     |                    |          |         |                      |                    |          |         |
| 1-Aminonaphthaline<br>(ng/24 hours)          | 1-aminonaphthalene                           | 110.77±43.30        | 5.08±2.81          | -95.41%  | <0.0001 | 93.27±55.58          | 5.34±3.55          | -94.28%  | 0.0006  |
| 2-Aminonaphthalene<br>(ng/24 hours)          | 2-aminonaphthalene                           | 27.37±12.01         | 2.00±1.22          | -92.70%  | <0.0001 | 27.55±14.39          | 2.15±1.01          | -92.20%  | 0.0003  |
| 4-Aminobiphenyl<br>(ng/24 hours)             | 4-aminobiphenyl                              | 21.53±7.35          | 4.73±3.01          | -78.02%  | <0.0001 | 19.45±9.32           | 4.46±2.45          | -77.07%  | 0.0003  |
| <i>o</i> -Toluidine (ng/24 hours)            | <i>o</i> -Toluidine                          | 203.39±87.49        | 85.09±59.23        | -58.17%  | <0.0001 | 204.36±125.18        | 67.70±25.90        | -66.70%  | 0.0055  |
| <b>Semi-volatile Organics</b>                |                                              |                     |                    |          |         |                      |                    |          |         |
| CEMA (µg/24 hours)                           | Acrylonitrile                                | 257.27±100.46       | 42.90±22.00        | -83.33%  | <0.0001 | 245.87±125.18        | 34.57±22.44        | -85.94%  | 0.0002  |
| HMPMA (µg/24 hours)                          | Crotonaldehyde                               | 515.22±213.14       | 98.01±47.80        | -80.98%  | <0.0001 | 521±64               | 97.18±53.90        | -81.37%  | 0.0006  |
| HPMA (µg/24 hours)                           | Acrolein                                     | 1.62±0.60           | 0.34±0.16          | -79.19%  | <0.0001 | 1.63±1.00            | 0.30±0.11          | -81.37%  | 0.0020  |
| MHBMA (µg/24 hours)                          | 1,3-butadiene                                | 2.91±2.02           | 0.13±0.09          | -95.52%  | <0.0001 | 2.47±1.80            | 0.14±0.05          | -94.51%  | 0.0022  |
| SPMA (µg/24 hours)                           | Benzene                                      | 5.34±3.11           | 0.31±0.21          | -94.10%  | <0.0001 | 4.70±3.15            | 0.34±0.21          | -92.86%  | 0.0014  |
| <b>Polycyclic Aromatic Hydrocarbon</b>       |                                              |                     |                    |          |         |                      |                    |          |         |
| 3-OH-B[a]P (pg/24 hours)                     | Benzo[a]pyrene                               | 240.68±323.64       | 139.32±437.19      | -42.11%  | 0.0420  | 218.70±217.70        | 61.38±43.61        | -71.94%  | 0.0233  |
| 3-OH-B[a]P (pg/24 hours)**                   | Benzo[a]pyrene                               | 240.68±323.64       | 66.16±62.79        | -72.51%  | 0.0015  | 218.70±217.70        | 61.38±43.61        | -71.94%  | 0.0117  |
| <b>TSNAs</b>                                 |                                              |                     |                    |          |         |                      |                    |          |         |
| Total NNAL (ng/24 hours)                     | 4-(methylnitrosamino)-1-(3-pyridyl)-butanol) | 668.71±318.51       | 275.41±165.32      | -58.81%  | <0.0001 | 600.59±404.24        | 286.51±166.15      | -52.30%  | 0.0062  |
| Total NNN (ng/24 hours)                      | N'-nitrosonornicotine                        | 11.82±11.88         | 2.06±1.40          | -82.54%  | <0.0001 | 63.45±165.86         | 2.11±0.80          | -96.68%  | 0.1131  |
| Total NNN (ng/24 hours)**                    | N'-nitrosonornicotine                        | 11.82±11.88         | 2.06±1.40          | -82.54%  | <0.0001 | 15.71±13.20          | 2.11±0.80          | -86.60%  | 0.0061  |
| <b>Nicotine</b>                              |                                              |                     |                    |          |         |                      |                    |          |         |
| Total nicotine equivalents<br>(mg/24 hours)* | Nicotine                                     | 17.05±6.44          | 12.12±7.75         | -28.91%  | 0.0010  | 16.79±8.80           | 0.65±1.10          | -96.12%  | <0.0001 |
| <b>Blood Biomarker (units)</b>               |                                              |                     |                    |          |         |                      |                    |          |         |
| Carboxyhemoglobin (%)                        | Carbon monoxide                              | 11.52±2.84          | 5.64±1.94          | -51.04%  | <0.0001 | 11.73±3.95           | 5.28±1.10          | -55.01%  | 0.0005  |

Abbreviations: CEMA = 2-cyanoethyl mercapturic acid, HMPMA = 3-hydroxy-1-methylpropyl-mercapturic acid, HPMA 3-hydroxypropyl mercapturic acid, MHBMA = monohydroxybutyl mercapturic acid, SPMA = S-phenyl mercapturic acid, 3-OH-B[a]P = 3-hydroxy-benzo[a]pyrene, TSNAs = tobacco specific nitrosamines, NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, NNN = N'-nitrosonornicotine, SD = standard deviation.

\* 24-hour urine total nicotine equivalents

\*\* Biomarker results excluding extreme value(s)

**Supplementary Table S3. Adverse Events reported with relationship to Vuse Solo across studies**

|                               | Campbell et al. 2021 | Hong et al. 2021 | Present Study |
|-------------------------------|----------------------|------------------|---------------|
| # Adverse Events              | 8                    | 9 <sup>a</sup>   | 36            |
| # of Subjects                 | 38                   | 9                | 21            |
| # Possibly Related to product | 0                    | 1                | 10            |
| # Related to product          | 0                    | 1                | 8             |

<sup>a</sup> Denotes only those adverse events that were captured during portions of the study when subjects used Vuse Solo

**Supplementary Table S4. Tobacco constituent and analytic methods**

| Urinary Biomarker                                             | Tobacco Constituent                          | Abbreviation  | Matrix      | Method     | LOD         | LLOQ        |
|---------------------------------------------------------------|----------------------------------------------|---------------|-------------|------------|-------------|-------------|
| 1-aminonaphthalene                                            | 1-aminonaphthalene                           | 1-AN          | Urine       | GC-MS      | 0.6 ng/L    | 1.8 ng/L    |
| 2-aminonaphthalene                                            | 2-aminonaphthalene                           | 2-AN          | Urine       | GC-MS      | 0.57 ng/L   | 1.7 ng/L    |
| 4-aminobiphenyl                                               | 4-aminobiphenyl                              | 4-ABP         | Urine       | GC-MS      | 0.51 ng/L   | 1.5 ng/L    |
| <i>o</i> -toluidine                                           | <i>o</i> -Toluidine                          | <i>o</i> -Tol | Urine       | GC-MS      | 0.83 ng/L   | 10 ng/L     |
| 2-cyanoethyl mercapturic acid                                 | Acrylonitrile                                | CEMA          | Urine       | LC-MS/MS   | 0.08 ng/mL  | 0.25 ng/mL  |
| 3-hydroxy-1-methylpropyl-mercapturic acid                     | Crotonaldehyde                               | HMPMA         | Urine       | LC-MS/MS   | 0.49 ng/mL  | 5 ng/mL     |
| 3-hydroxypropyl mercapturic acid                              | Acrolein                                     | HPMA          | Urine       | LC-MS/MS   | 12.58 ng/mL | 25 ng/mL    |
| Monohydroxybutyl mercapturic acid                             | 1,3-butadiene                                | MHBMA         | Urine       | LC-MS/MS   | 0.029 ng/mL | 0.129 ng/mL |
| S-phenyl mercapturic acid                                     | Benzene                                      | SPMA          | Urine       | LC-MS/MS   | 5.0 pg/mL   | 0.02 ng/mL  |
| 3-hydroxy-benzo[a]pyrene                                      | Benzo[a]pyrene                               | 3-OH-B[a]P    | Urine       | LC-MS/MS   | 33.3 fg/mL  | 100 fg/mL   |
| 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol) + glucuronides | 4-(methylnitrosamino)-1-(3-pyridyl)-butanol) | Total NNAL    | Urine       | LC-MS/MS   | 2 pg/mL     | 5 pg/mL     |
| N'-nitrosonornicotine + glucuronides                          | N'-nitrosonornicotine                        | Total NNN     | Urine       | LC-MS/MS   | 0.8 pg/mL   | 2 pg/mL     |
| Total nicotine equivalents                                    |                                              | NicEq-T*      | Urine       | Calculated |             |             |
| Nicotine                                                      | Unconjugated Nicotine                        | Nic-U         | Urine       | LC-MS/MS   | 0.5 ng/L    | 1.5 ng/L    |
| Cotinine                                                      | Unconjugated Cotinine                        | Cot-U         | Urine       | LC-MS/MS   | 0.17 ng/L   | 0.51 ng/L   |
| Cotinine                                                      | Unconjugated trans-3'-hydroxycotinine        | OH-Cot        | Urine       | LC-MS/MS   | 0.21 ng/L   | 0.63 ng/L   |
| Nicotine                                                      | Nicotine-N-glucuronide                       | Nic-Gluc      | Urine       | LC-MS/MS   | 0.17 ng/L   | 0.51 ng/L   |
| Cotinine                                                      | Cotinine-N-glucuronide                       | Cot-Gluc      | Urine       | LC-MS/MS   | 0.33 ng/L   | 0.99 ng/L   |
| Metabolite of trans-3'-hydroxycotinine                        | Trans-3'-hydroxycotinine-O-glucuronide       | OH-Cot-Gluc   | Urine       | LC-MS/MS   | 0.75 ng/L   | 2.25 ng/L   |
| <b>Blood Biomarker</b>                                        |                                              |               |             |            |             |             |
| Carboxyhemoglobin                                             | Carbon monoxide                              | COHb          | Whole Blood | HS-GC-MS   | 0.40%       | 0.75%       |

Abbreviations: GC = gas chromatography, MS = mass spectrometry, LC = liquid chromatography, HS = headspace, LOD = limit of detection, LLOQ = lower limit of quantification.

\*NicEq-T = Nic-U and the 5 metabolites converted to molar Nic-U equivalents and summed.