**Supplementary Table 1|** pH measurements of the exposure conditions for the TRPA1 inhibitor studies.

|  |  |
| --- | --- |
| **Exposure condition** | **pH** |
| **Media-only** | 7.50 |
| **DMSO** | 7.48 |
| **AP-18 (10 µM)** | 7.47 |
| **AP-18 (10 µM) + 1% Cinnamon** | 7.52 |
| **1% Cinnamon** | 7.53 |



**Supplementary Figure 1| A) Cell count B) % of viable cells, and C) LDH release analysis for AP-18 (10μM) and cinnamaldehyde (2.5, 5, and 10 mM).** A one-way ANOVA with multiple comparisons, corrected using Tukey was used to determine statistical significance. Data are expressed as mean S.E.M (n=6 per group). \**p*<0.05; *\*\*p*<0.01; \*\*\**p*<0.001; \*\*\*\**p*<0.0001 vs control; #*p*<0.05; ##*p*<0.01; ###*p*<0.001; ####*p*<0.0001 vs AP-18 (10 µM) + cin. All groups were significantly different compared to the 0.1% Triton X-100 control.

**Supplementary Figure 2**

**A**

A picture containing text, screenshot, diagram, plot

Description automatically generated

**Supplementary Figure 2| Comprehensive gene stability of the housekeeping genes tested for the conditions of our study.** Only GAPDH was not affected by AP-18 (10 µM) alone and AP-18 (10 µM) + cinnamon exposure. the other housekeeping genes, namely HPRT1, beta-actin, and 18s rRNA, was significantly affected by the AP-18 and Cinnamon treatments. These findings suggest that these genes may not be suitable as reference genes under the specific conditions of our study

**Supplementary Table 2** | Cell count analysis for all 53 flavored e-liquids vs. media-only controls and their respective PG/VG controls.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **E-liquid name** | | **Flavors vs. media-only control** | | | **Correlation vs PG/VG controls (R2)** | **Simple Linear regression (R2)** | **PG/VG ratio** | **Flavour Category** | **Manufacturer** |
| **0.25%** | **0.5%** | **1%** |  |  |  |  |  |
| 1 | **Apple** | <0.0001 | <0.0001 | <0.0001 | 0.9848 | 0.9724 | **60/40** | **Fruit**/**Other** | **V4POUR** |
| 2 | **Artic Fruit** | 0.0268 | <0.0001 | <0.0001 | 0.9748 | 0.9853 | **60/40** | **Fruit**/**Citrus** | **VAMPIRE VAPE** |
| 3 | **Anised** | 0.0004 | 0.0002 | <0.0001 | 0.9868 | 0.8992 | **60/40** | **Spices** | **V4POUR** |
| 4 | **Heisenberg** | 0.0026 | <0.0001 | <0.0001 | 0.9824 | 0.9926 | **60/40** | **Candy** | **VAMPIRE VAPE** |
| 5 | **Rollie** | ns | <0.0001 | <0.0001 | 0.9560 | 0.9891 | **60/40** | **Tobacco** | **V4POUR** |
| 6 | **Cool Cig** | ns | ns | <0.0001 | 0.9082 | 0.6990 | **60/40** | **Tobacco** | **V4POUR** |
| 7 | **Cool Mint** | <0.0001 | <0.0001 | <0.0001 | 0.9642 | 0.9774 | **60/40** | **Menthol**/  **Mint** | **V4POUR** |
| 8 | **Energy Drink** | <0.0001 | <0.0001 | <0.0001 | 0.9875 | 0.9719 | **60/40** | **Other** **Beverages** | **V4POUR** |
| 9 | **Wine Gummies** | <0.0001 | <0.0001 | <0.0001 | 0.9965 | 0.9700 | **60/40** | **Candy** | **V4POUR** |
| 10 | **Butterscotch** | <0.0001 | <0.0001 | <0.0001 | 0.9735 | 0.9891 | **60/40** | **Other** **Sweets** | **V4POUR** |
| 11 | **Grape** | 0.0019 | <0.0001 | <0.0001 | 0.9944 | 0.9695 | **60/40** | **Fruit**/**Other** | **V4POUR** |
| 12 | **NRG Charger** | <0.0001 | <0.0001 | <0.0001 | 0.9760 | 0.9617 | **60/40** | **Other** **Beverages** | **V4POUR** |
| 13 | **Virginia** | <0.0001 | <0.0001 | <0.0001 | 0.9992 | 0.9937 | **60/40** | **Tobacco** | **V4POUR** |
| 14 | **Lemon & Lime** | <0.0001 | <0.0001 | <0.0001 | 0.9886 | 0.9643 | **60/40** | **Fruit**/**Citrus** | **V4POUR** |
| 15 | **Black jack** | <0.0001 | <0.0001 | <0.0001 | 0.9793 | 0.9692 | **60/40** | **Candy** | **V4POUR** |
| 16 | **Blueberry** | 0.0043 | <0.0001 | <0.0001 | 0.9311 | 0.9774 | **60/40** | **Fruit**/**Berries** | **V4POUR** |
| 17 | **Raspberry** | ns | <0.0001 | <0.0001 | 0.9992 | 0.8997 | **60/40** | **Fruit**/**Berries** | **VAMPIRE VAPE** |
| 18 | **Rhubarb Crumble** | ns | <0.0001 | <0.0001 | 0.9222 | 0.9833 | **60/40** | **Dessert** | **V4POUR** |
| 19 | **Banana** | ns | ns | 0.0015 | 0.8608 | 0.7071 | **60/40** | **Fruit**/**Tropical** | **V4POUR** |
| 20 | **Cola** | ns | 0.0016 | <0.0001 | 0.9405 | 0.9769 | **60/40** | **Other** **Beverages** | **V4POUR** |
| 21 | **Toffee** | ns | 0.0052 | <0.0001 | 0.9624 | 0.9555 | **60/40** | **Other** **Sweets** | **V4POUR** |
| 22 | **Custard** | 0.0010 | <0.0001 | <0.0001 | 0.9922 | 0.9634 | **60/40** | **Dessert** | **V4POUR** |
| 23 | **Coffee** | <0.0001 | <0.0001 | <0.0001 | 0.9778 | 0.9848 | **60/40** | **Coffee**/**Tea** | **V4POUR** |
| 24 | **Menthol** | 0.0003 | 0.0041 | <0.0001 | 0.8075 | 0.6661 | **60/40** | **Menthol**/**Mint** | **VAMPIRE VAPE** |
| 25 | **Peach** | ns | 0.0005 | <0.0001 | 0.9955 | 0.8196 | **60/40** | **Fruit**/**Other** | **V4POUR** |
| 26 | **Pineapple** | <0.0001 | <0.0001 | <0.0001 | 0.7459 | 0.7607 | **60/40** | **Fruit**/**Other** | **V4POUR** |
| 27 | **Mint** | <0.0001 | <0.0001 | <0.0001 | 0.9562 | 0.9694 | **60/40** | **Menthol**/**Mint** | **V4POUR** |
| 28 | **Bat Juice** | ns | ns | <0.0001 | 0.9204 | 0.9321 | **60/40** | **Fruit**/**Berries** | **V4POUR** |
| 29 | **Charger** | <0.0001 | <0.0001 | <0.0001 | 0.9034 | 0.8644 | **60/40** | **Fruit**/**Berries** | **VAMPIRE VAPE** |
| 30 | **Fruit Salad** | <0.0001 | <0.0001 | <0.0001 | 0.9703 | 0.9595 | **60/40** | **Fruit** | **V4POUR** |
| 31 | **Menthol Tobacco** | 0.0319 | <0.0001 | <0.0001 | 0.9972 | 0.9937 | **60/40** | **Crossed** **Flavours** | **VAMPIRE VAPE** |
| 32 | **Vanilla tobacco\*** | <0.0001 | <0.0001 | <0.0001 | 0.8309 | 0.7666 | **60/40** | **Crossed** **Flavours** | **VAMPIRE VAPE** |
| 33 | **Strawberry Menthol** | <0.0001 | <0.0001 | <0.0001 | 0.9676 | 0.9612 | **60/40** | **Crossed** **Flavours** | **V4POUR** |
| 34 | **Vanilla** | <0.0001 | <0.0001 | <0.0001 | 0.9995 | 0.9869 | **60/40** | **Other** **Sweets** | **VAMPIRE VAPE** |
| 35 | **Belgian Cocoa** | ns | ns | <0.0001 | 0.9897 | 0.8641 | **PG only** | **Fruit**/**Tropical** | **V4POUR** |
| 36 | **Menthol Ice** | ns | ns | ns | 0.9960 | 0.8869 | **PG only** | **Menthol**/**Mint** | **V4POUR** |
| 37 | **Turkish Tobacco** | ns | ns | 0.0020 | 0.9999 | 0.9642 | **PG only** | **Tobacco** | **V4POUR** |
| 38 | **Black Diamond** | ns | ns | 0.0001 | 1.000 | 0.9656 | **PG only** | **Unknown** | **Vapeshop** |
| 39 | **Café Mocha** | ns | <0.0001 | <0.0001 | 0.9382 | 0.8537 | **PG only** | **Coffee**/**Tea** | **V4POUR** |
| 40 | **Mango** | 0.0006 | <0.0001 | <0.0001 | 0.9885 | 0.9352 | **60/40** | **Fruit** | **Vapemate** |
| 41 | **Cappuccino** | 0.0010 | <0.0001 | <0.0001 | 0.9684 | 0.9798 | **60/40** | **Coffee**/**Tea** | **Vapeshop** |
| 42 | **Dark rum** | 0.0005 | <0.0001 | <0.0001 | 0.8137 | 0.7583 | **60/40** | **Alcohol** | **Motivape** |
| 43 | **Cinnamon\*** | <0.0001 | <0.0001 | <0.0001 | 0.7891 | 0.9123 | **70/30** | **Spices** | **Vapekit** |
| 44 | **Mojito** | 0.0001 | <0.0001 | <0.0001 | 0.9452 | 0.9366 | **60/40** | **Alcohol** | **Vapekit** |
| 45 | **Watermelon** | <0.0001 | <0.0001 | <0.0001 | 0.9510 | 0.7412 | **60/40** | **Fruit**/**Other** | **Vampirevape** |
| 46 | **Coconut** | ns | <0.0001 | <0.0001 | 0.9996 | 0.8840 | **60/40** | **Fruit**/**Tropical** | **Vapemate** |
| 47 | **Hazelnut\*** | <0.0001 | <0.0001 | <0.0001 | 0.8428 | 0.7477 | **50/50** | **Nuts** | **Mist** |
| 48 | **Energy** | 0.0004 | <0.0001 | <0.0001 | 0.9736 | 0.9930 | **60/40** | **Other** **Beverages** | **VAMPIRE VAPE** |
| 49 | **Cherry, mint & menthol** | 0.0057 | <0.0001 | <0.0001 | 0.9401 | 0.8494 | **60/40** | **Crossed** **Flavours** | **Vapourlites** |
| 50 | **Blueberry forest** | 0.0012 | <0.0001 | <0.0001 | 0.9996 | 0.9741 | **60/40** | **Fruit**/**Berries** | **Vapourlites** |
| 51 | **Almond** | 0.0010 | <0.0001 | <0.0001 | 0.9969 | 0.9741 | **60/40** | **Nuts** | **Nucig** |
| 52 | **Lemon, Strawberry & Anised** | <0.0001 | <0.0001 | <0.0001 | 0.9626 | 0.9953 | **60/40** | **Crossed** **Flavours** | **Vapourlites** |
| 53 | **Strawberry, vanillin & Custard** | <0.0001 | <0.0001 | <0.0001 | 0.9986 | 0.9521 | **60/40** | **Crossed** **Flavours** | **Vapourlites** |

**Supplementary Table 3|** LDH analysis for all 53 flavored e-liquids vs. media-only controls and their respective PG/VG controls.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **E-liquid name** | | **Flavors vs media-only control** | | | **Correlation vs PG/VG controls (R2)** | **Simple Linear regression (R2)** | **Flavour Category** |
| **0.25%** | **0.5%** | **1%** |  |  |  |
| 1 | **Apple** | ns | ns | < 0.05 | 0.857 | 0.08624 | **Fruit**/**Other** |
| 2 | **Artic Fruit** | ns | ns | < 0.01 | 0.923 | 0.8288 | **Fruit**/**Citrus** |
| 3 | **Anised** | ns | ns | ns | 0.999 | 0.1957 | **Spices** |
| 4 | **Heisenberg** | ns | ns | < 0.0001 | 0.999 | 0.6662 | **Candy** |
| 5 | **Rollie** | ns | ns | < 0.0001 | 0.675 | 0.5542 | **Tobacco** |
| 6 | **Cool Cig** | ns | ns | < 0.0001 | 0.981 | 0.1216 | **Tobacco** |
| 7 | **Cool Mint** | ns | ns | < 0.05 | 0.810 | 0.3988 | **Menthol**/**Mint** |
| 8 | **Energy Drink** | ns | ns | < 0.0001 | 0.194 | 0.8752 | **Other** **Beverages** |
| 9 | **Wine Gummies** | ns | < 0.05 | < 0.001 | 0.874 | 0.3222 | **Candy** |
| 10 | **Butterscotch** | ns | ns | ns | 0.887 | 0.3043 | **Other** **Sweets** |
| 11 | **Grape** | ns | < 0.001 | < 0.0001 | 0.974 | 0.6702 | **Fruit**/**Other** |
| 12 | **NRG Charger** | ns | ns | < 0.0001 | 0.996 | 0.7080 | **Other** **Beverages** |
| 13 | **Virginia** | ns | ns | < 0.001 | 0.999 | 0.3545 | **Tobacco** |
| 14 | **Lemon & Lime** | ns | ns | < 0.001 | 0.999 | 0.5448 | **Fruit**/**Citrus** |
| 15 | **Black jack** | ns | ns | < 0.001 | 0.999 | 0.7071 | **Candy** |
| 16 | **Blueberry** | ns | < 0.05 | < 0.001 | 0.9999 | 0.1982 | **Fruit**/**Berries** |
| 17 | **Raspberry** | ns | < 0.01 | < 0.001 | 0.9633 | 0.6978 | **Fruit**/**Berries** |
| 18 | **Rhubarb Crumble** | ns | < 0.01 | < 0.001 | 0.9739 | 0.5255 | **Dessert** |
| 19 | **Banana** | ns | ns | < 0.01 | 0.3958 | 0.4489 | **Fruit**/**Tropical** |
| 20 | **Cola** | ns | ns | < 0.001 | 0.1568 | 0.9769 | **Other** **Beverages** |
| 21 | **Toffee** | ns | ns | ns | 0.1242 | 0.9555 | **Other** **Sweets** |
| 22 | **Custard** | ns | ns | ns | 0.7625 | 0.1115 | **Dessert** |
| 23 | **Coffee** | ns | < 0.05 | < 0.0001 | 0.9854 | 0.9100 | **Coffee**/**Tea** |
| 24 | **Menthol** | ns | ns | ns | 0.155 | 0.550 | **Menthol**/**Mint** |
| 25 | **Peach** | ns | ns | ns | 0.762 | 0.712 | **Fruit**/**Other** |
| 26 | **Pineapple** | ns | ns | < 0.001 | 0.867 | 0.547 | **Fruit**/**Other** |
| 27 | **Mint** | ns | ns | < 0.001 | 0.998 | 0.847 | **Menthol**/**Mint** |
| 28 | **Bat Juice** | ns | ns | ns | 0.997 | 0.381 | **Fruit**/**Berries** |
| 29 | **Charger** | ns | ns | ns | 0.366 | 0.287 | **Fruit**/**Berries** |
| 30 | **Fruit Salad** | < 0.05 | < 0.05 | < 0.0001 | 0.933 | 0.611 | **Fruit** |
| 31 | **Menthol Tobacco** | < 0.05 | < 0.001 | < 0.0001 | 0.997 | 0.705 | **Crossed** **Flavours** |
| 32 | **Vanilla tobacco\*** | < 0.0001 | < 0.0001 | < 0.0001 | 0.772 | 0.556 | **Crossed** **Flavours** |
| 33 | **Strawberry Menthol** | < 0.001 | < 0.001 | < 0.001 | 0.997 | 0.701 | **Crossed** **Flavours** |
| 34 | **Vanilla** | < 0.001 | < 0.001 | < 0.001 | 0.906 | 0.721 | **Other** **Sweets** |
| 35 | **Belgian Cocoa** | ns | ns | ns | -0.342 | 0.0303 | **Fruit**/**Tropical** |
| 36 | **Menthol Ice** | ns | ns | ns | 0.593 | 0.312 | **Menthol**/**Mint** |
| 37 | **Turkish Tobacco** | ns | ns | ns | -0.187 | 0.0921 | **Tobacco** |
| 38 | **Black Diamond** | ns | ns | ns | -0.820 | 0.0921 | **Unknown** |
| 39 | **Café Mocha** | ns | ns | ns | 0.643 | 0.443 | **Coffee**/**Tea** |
| 40 | **Mango** | ns | ns | ns | -0.576 | 0.769 | **Fruit** |
| 41 | **Cappuccino** | ns | ns | < 0.0001 | -0.460 | 0.820 | **Coffee**/**Tea** |
| 42 | **Dark rum** | ns | ns | ns | -0.954 | 0.115 | **Alcohol** |
| 43 | **Cinnamon\*** | < 0.0001 | < 0.0001 | < 0.0001 | -0.3244 | 0.8863 | **Spices** |
| 44 | **Mojito** | ns | ns | <0.0001 | -0.0695 | 0.7117 | **Alcohol** |
| 45 | **Watermelon** | ns | ns | ns | -0.4978 | 0.5519 | **Fruit**/**Other** |
| 46 | **Coconut** | ns | ns | ns | -0.9408 | 0.0375 | **Fruit**/**Tropical** |
| 47 | **Hazelnut\*** | < 0.001 | < 0.001 | < 0.001 | 0.930 | 0.569 | **Nuts** |
| 48 | **Energy** | < 0.05 | < 0.05 | < 0.001 | 0.694 | 0.144 | **Other** **Beverages** |
| 49 | **Cherry, mint & menthol** | ns | ns | ns | -0.302 | 0.849 | **Crossed** **Flavours** |
| 50 | **Blueberry forest** | ns | ns | ns | -0.534 | 0.995 | **Fruit**/**Berries** |
| 51 | **Almond** | ns | ns | <0.0001 | -0.576 | 0.630 | **Nuts** |
| 52 | **Lemon, Strawberry & Anised** | ns | ns | < 0.05 | -0.343 | 0.952 | **Crossed** **Flavours** |
| 53 | **Strawberry, vanillin & Custard** | ns | ns | < 0.0001 | -0.486 | 0.981 | **Crossed** **Flavours** |

**Supplementary Table 4|** GeNorm analysis for the housekeeping genes testes

A screenshot of a document

Description automatically generated with low confidence

**Supplementary Table 5 | Raw data of the qPCR of GADPH and TRPA1 following treatment to AP-18 and cinnamon e-liquid.**

