Key Variables Important in Accessing E-Liquids

Components, Flavors, Aerosols and Scrutinizing the Scientific Literature

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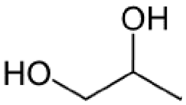
Outline of talk

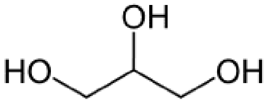
- E-Liquids, components, formulations
  - Carrier liquids for e-liquid “base”
  - Nicotine
  - Flavors
    - Compounds of current inhalation concern
  - Quantitative determination challenges
- Thermal decomposition of e-liquids
  - High emissions: 2nd Generaton Devices (CE4 top-coil, some bottom-coil)
  - Very low emissions: newer 3rd Generation devices
- Scrutinizing EC literature (possible red flags)

E-liquid components and formulations

- **Carrier liquids**
  - **Propylene Glycol (PG, 1,2-propanediol)**



    - Should be USP grade
    - Lower viscosity than VG (BP 188.2 °C)
    - Better wicking than VG
    - Tends to dehydrate mouth and throat ("drink lots of water!")
    - Some people are extremely sensitive to PG: chapped lips, mouth sores, gastric problems, some rashes (actual allergy) reported.
  - **Vegetable Glycerin (VG, 1,2,3-propanetriol)**



    - Should be USP grade, not Food Grade or lower purity
    - Very viscous, more "vapor" (visible aerosol) than PG, sweetener
    - BP 290 °C
    - Needs thinning (PG, H_2O) to wick well
    - Less dehydrating than PG for most people

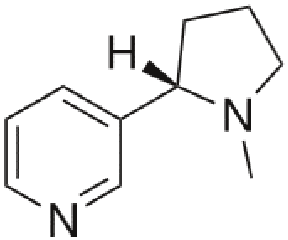
E-liquid components and formulations

- **Typical carrier "base" formulations**
  - Early days: 100% PG, but VG grew in popularity as an additive to or replacement for PG
  - Now most common is probably 60:40 PG:VG (volume ratio)
    - Other ratios also available, depending on user's preference
  - VG only bases (no PG, for those that are PG-sensitive)
    - Typically needs 10-20% water to wick well
      - 90:10 VG:H_2O BP 140 ^oC
    - Used to avoid the chronic dry mouth common to PG vaping

E-liquid components and formulations

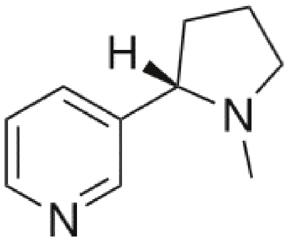
- **Newer carrier compound: 1,3-propanediol (PDO or 1,3-PDO)**
  - BP 211-217 ^{o}C
  - Similar thermal stability as PG and VG
  - Available in USP Grade
    - No clear inhalation risks…thus far.
    - Long-term health risks from inhalation are unknown
  - Viscosity similar to PG, similar wicking
  - Less oral/throat dehydration than PG
  - Not as sweet as VG, has a slight plastic taste
  - Not common currently

E-liquid components and formulations



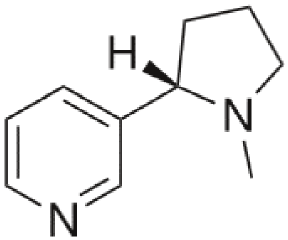
- **Nicotine (free-base)**
  - AEMSA: USP grade or higher purity
  - USP grade is used for NRTs (patch, gum, lozenge)
    - Assumes extraction from tobacco (l-nicotine enantiomer)
    - 99+% pure (anhydrous titration)
    - Maximum 1% impurities
      - Other tobacco alkaloids (myosmine, nornicotine, etc)
      - May contain trace TSNAs
      - Nicotine N-oxides (air oxidation)
    - Maximum 10 ppm heavy metals
    - Maximum 1 ppm arsenic

E-liquid components and formulations



- **Nicotine**
  - **Commonly used nicotine for e-liquids currently can far exceed USP purity**
    - Minimum USP grade would taste harsh (common complaint in early years of e-cigs)
    - Extracted nicotine is distilled under inert gas (much higher purity)
    - "Tasteless" or "invisible" nicotine
      - Far less harshness, nasal tingle on exhale
      - At typical nicotine concentrations very little nicotine sensory "feedback"
  - **Synthetic nicotine (non-tobacco nicotine)**
    - Racemic (50:50 l:d)
    - More expensive than extracted nicotine from tobacco
    - Long-term use of d-nicotine unknown in humans
    - Currently of only limited availability
    - Exempt from FDA regulations, since not a tobacco product?

E-liquid components and formulations



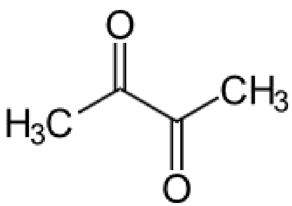
- **Nicotine**
  - **Nicotine concentrations in e-liquid for long-term users**
    - Early days (~5-9 Watts): average 12 mg/mL (Farsalinos, 2013)
    - Current is lower (higher wattage): average 6 mg/mL (Etter, 2016)
      - Higher efficiency of current devices
        - 62% used 3rd generation devices
        - More aerosol production plus deeper lung penetration
        - Users are tending to naturally lower nicotine concentration with high-efficiency 3rd generation devices.
  - **Nicotine salt solutions (protonated nicotine, nicotinium)**
    - Dominant form in tobacco
    - Weak acids (benzoic, pyruvic, citric) create salt forms in e-liquids
    - Meant to more mimic tobacco experience
    - Less harsh than typical free-base form.

E-liquid components and formulations

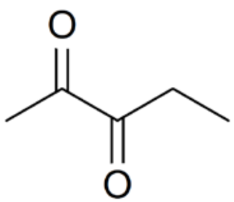
- **Flavors**
  - **Generally Recognized as Safe (GRAS) food flavorings**
    - Concentrations in e-liquids generally ≤15% by volume, depending on flavor, strength of pure flavoring, etc.
    - Very complex mixture of dozens of compounds
      - Example: strawberry can be 50-100 compounds
    - “Pure” flavor is usually flavor compounds in PG, VG, ethanol, water, or triacetin (or mixtures of these)
    - Considered safe for *ingestion*, largely unknown for *inhalation*

E-liquid components and formulations

- **Flavors**
  - **Some flavor compounds may be toxic or irritating with inhalation**

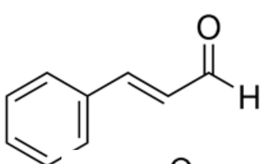


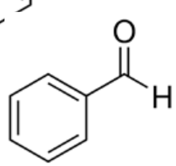
DA



AP

- α-Diketones (butter, cream, DA: diacetyl, AP: acetylpropionyl)
  - Lung damage known in humans (Harber, 2006) and rodents (Hubbs, 2012).
  - Bronchiolitis obliterans (“Popcorn lung”)
    - Rare, but irreversible lung scarring and necrosis
  - Farsalinos *et al, NTR,* 2015:
    - 74% of sweet e-liquids (*N* = 150) positive for DA and/or AP
    - Some very high, higher than NIOSH work limits
    - On average lower than smoking (combustion products)
  - More recent, Allan *et al,* 2015:
    - Still present, but *much* lower on average than a year earlier
    - Average *much* lower than smoking

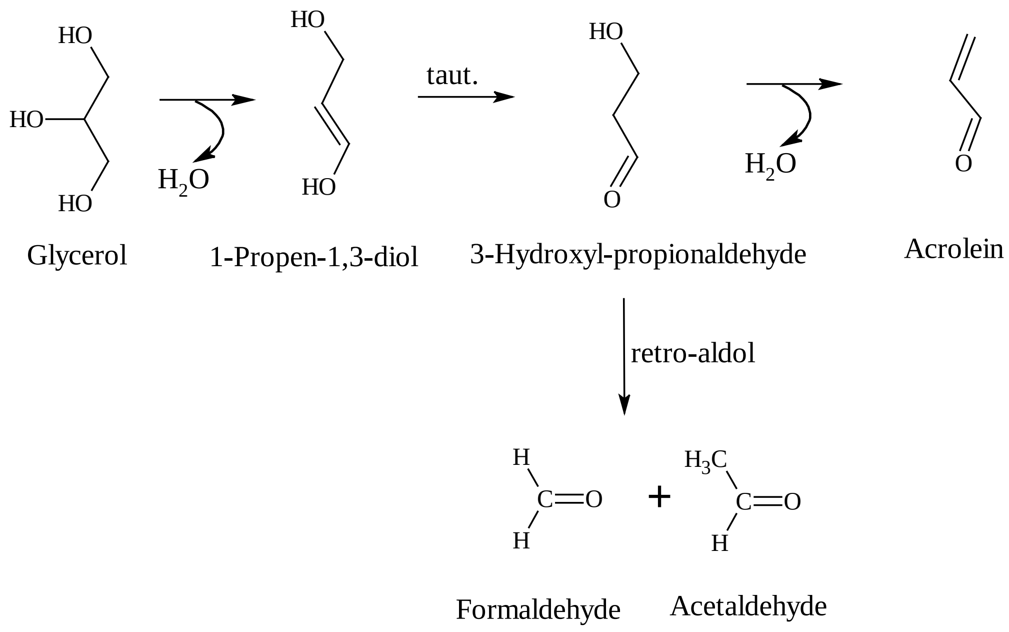




- Cinnamaldehyde (hot cinnamon, irritant, maybe cytotoxic)
- Benzaldehyde (cherry, almond)
  - Recently highly publicized
  - Probably not a serious problem. >>Less than workplace limits.

Thermal Decomposition of E-Liquids

- Thermal decomposition of glycerol produces (among other compounds) formaldehyde, acetaldehyde and acrolein.
- Free-radical loss of H_2O with additional retro-aldol reaction possible at higher temperature. (Paine, 2007)



- Propylene glycol produces these too, plus propionaldehyde.
- Since early 2015, at least four highly publicized studies showing extremely high aldehyde emissions from older EC devices (mainly CE4).

"Effect of Variable Vower Levels on the Yield of Total Aerosol Mass and Formation of Aldehydes in E-Cigarette Aerosols"
Gillman, Kistler, *et al.,* 2016

Carbonyl Analysis with High Performance Liquid Chromatography (HPLC):

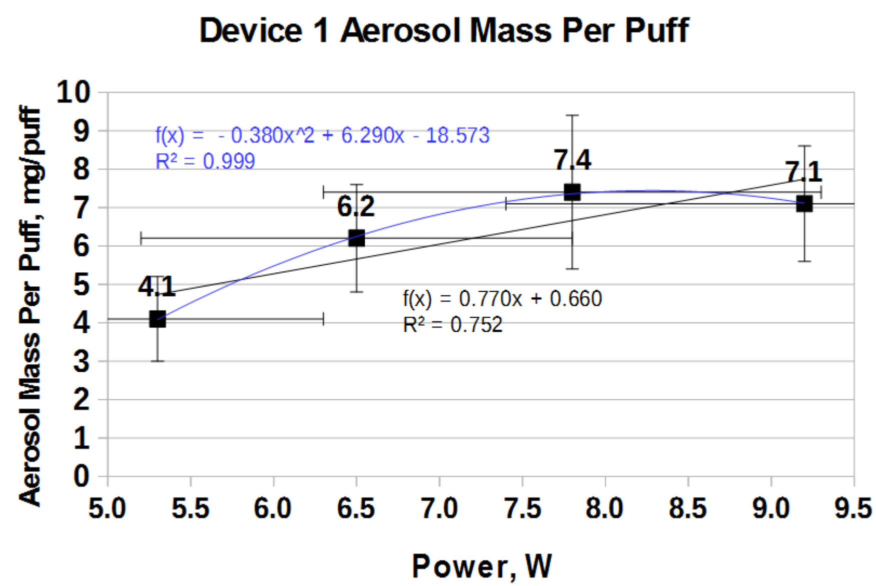
- Derivitized HPLC, modified method by CORESTA (No. 75, CORESTA 2011)
- EC aerosol passed through impinger containing 35 mL 2,4-dinitrophenylhydrazine (DNPH) trapping solution. 5 mL of this is pyridine-quenched and analyzed.
- HPLC-UV (Agilent Model 1100). Limit of detection (LOD) = 0.015 μg/mL for all carbonyl compounds.
- Transient acetals, hemiacetals, and hydrates of all carbonyl compounds are also trapped by DNPH, and cannot be differentiated from the free-aldehydes.
- Method is commonly used for air sampling of carbonyls, without differentiation from acetals, hemiacetals, or hydrates.

EC Liquid, Puffing Protocol, Mass Analysis and Repetition Parameters:

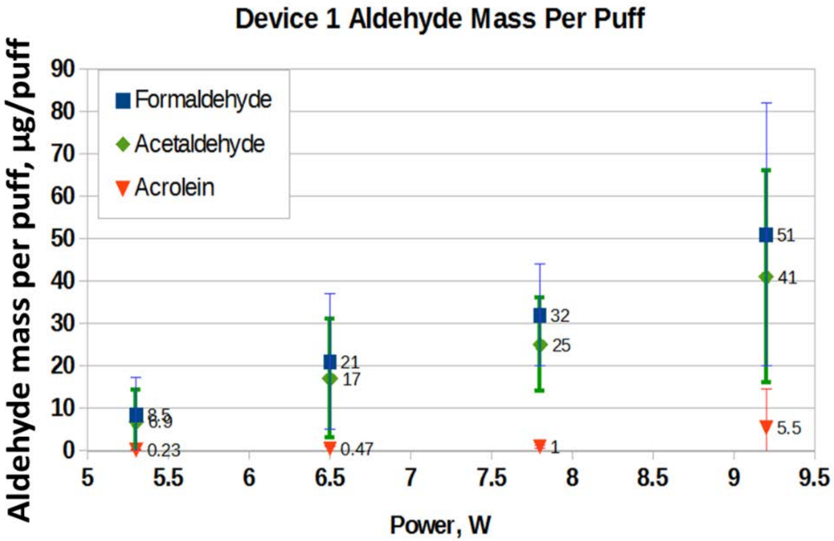
- E-liquid (unflavored): 48% propylene glycol, 50% glycerol, 2% nicotine, wt/wt.
- Puffs: 4 sec duration, square wave, 55 mL aerosol volume generated per puff.
- Each device was puffed 25 times (1 puff-block), with 30 sec separation, mass lost was recorded, and DNPH trapping solution analyzed (N=6 blocks, 150 puffs).
- Atomizer or coil-heads were new, and e-liquid fresh, for each puff-block.
- Power supply was fully charged for each puff-block.
- Three 2^{nd}-generation and two 3^{rd}-generation atomizer devices investigated.

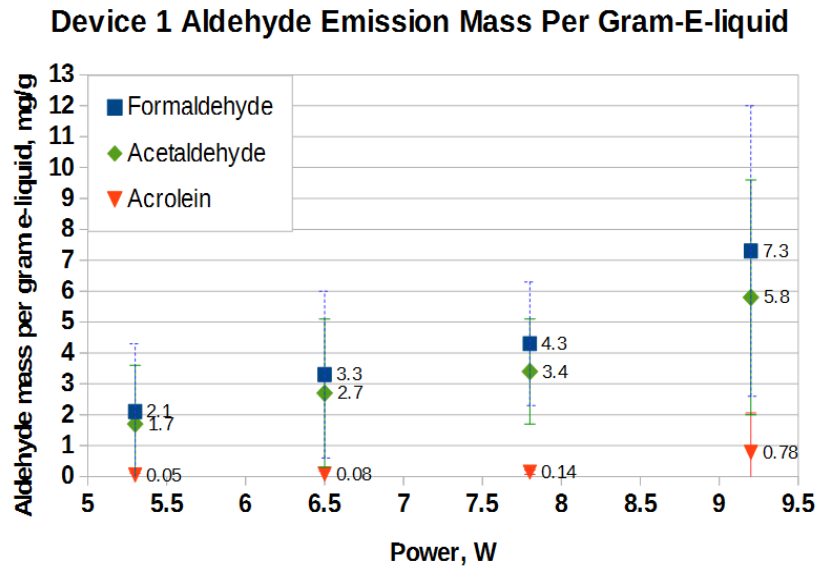
CE4 Top-coil Clearomizer
(2.8 ohm, Power: iTaste VV4, 5.3 – 9.2 W)

- Older **2nd-generation design**, known for poor performance and "dry-puff", due to wick not replenishing hot coil with liquid fast enough (Farsalinos *et al.*, *Addiction*, 2015).
- *Largely unpopular with users now for this reason, for years.*
- Favored in recently publicized high-aldehyde emission studies.
- Production drops above 7.8W as *decomposition increases*. Estimated maximum: 8.3W.
- Large variance of performance and power between units, indicating low quality control. *Not recommended for current emissions studies.*



CE4 ALDEHYDE EMISSIONS, MASS PER PUFF AND PER GRAM E-LIQUID





***CE4 formaldehyde emissions exceeded both smoking exposure and OSHA workday limits for all power settings*, as did acrolein at the highest power. Emissions from power levels >8W likely produces noxious and intolerable dry-puff, explaining general experienced consumer aversion to this device.**

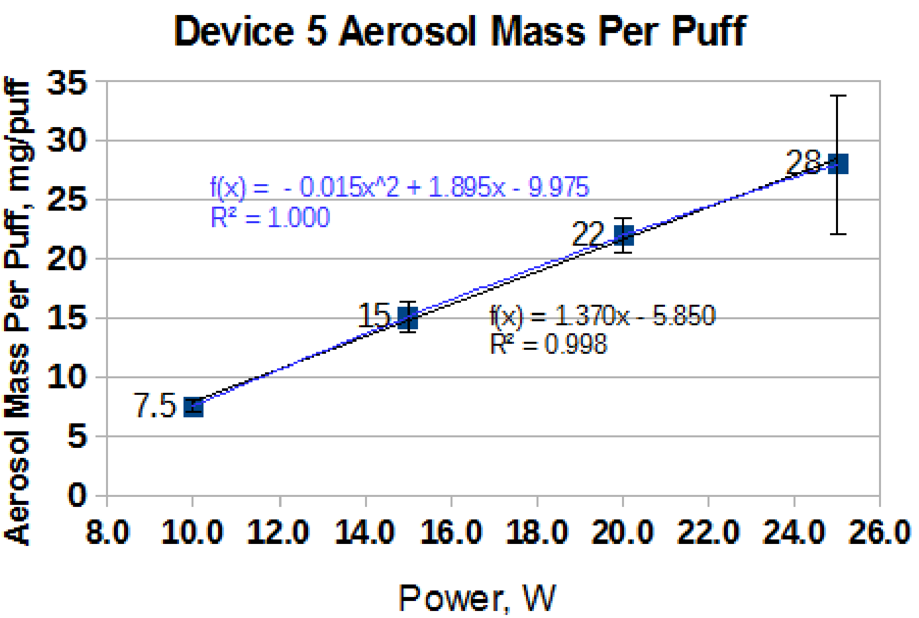
Cigarette (1 pack) aldehyde exposure (Counts 2005):

- Formaldehyde: 1.5-2.5 mg
- Acetaldehyde: 10-30 mg
- Acrolein: 1.5-3.0 mg

OSHA 8-hr workday exposure limits[8] (est.):

- Formaldehyde: 5.3 mg
- Acetaldehyde: 2088 mg
- Acrolein: 1.5 mg

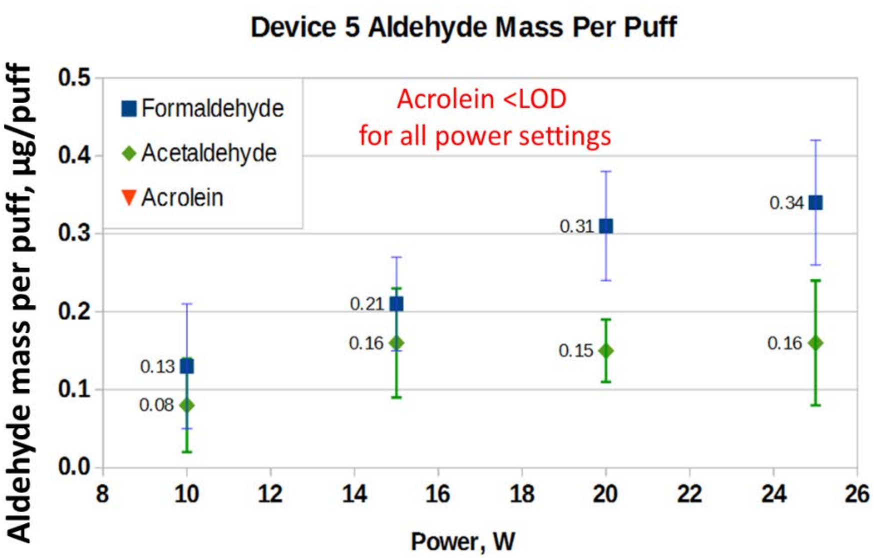
3rd-Generation Atomizer
Bottom-single-coil (Subtank, Kanger), 0.72Ω
Power: DNA-40 (Evolv) (10.0 – 25.0 W)

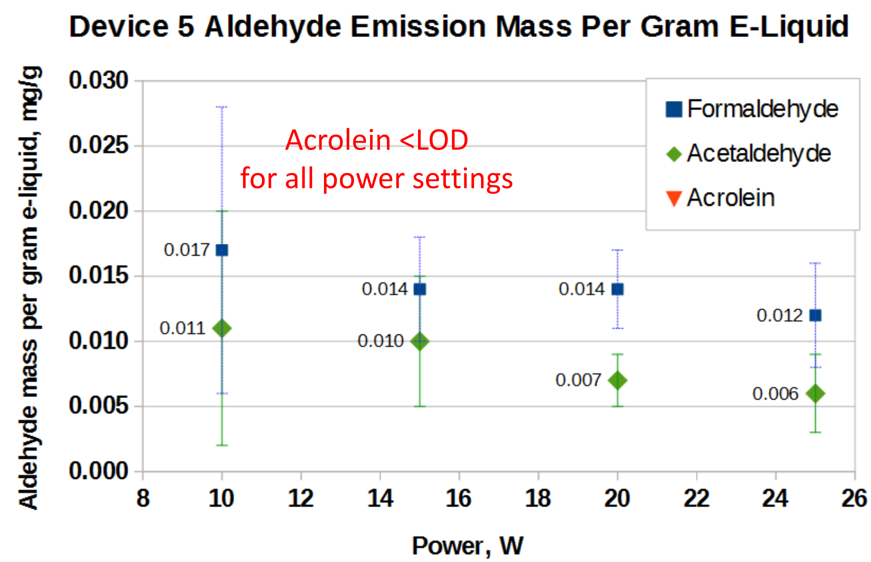


"Subohm" (coil resistance < 1Ω) 3rd-gen device for efficient high-wattage vaping (> 20 W). Large gauge coil wire, cotton wick, immersed coil-head. Air-flow control.

Quadratic production dependence, but close to linear to 25W. Low production variance at all wattages until 25W. Estimated maximum: 63.2W. Supports low decomposition.

SUBTANK ALDEHYDE EMISSIONS, MASS PER PUFF AND PER GRAM E-LIQUID





- 3rd Gen Subtank had no acrolein measured, and formaldehyde and acetaldehyde emissions are extremely low, and *decrease* on a per-gram basis with increased power, similar to Device 4.
- ***Formaldehyde and acetaldehyde emissions are <1% of smoking and OSHA, and about 0.1% of CE4, even at 25W.***
- 3rd-generation device popularity and efficacy further supported.
- Other 3rd Generation atomizers showing very low CO emissions
  - Aspire Nautilus (unpublished)
  - Joyetech Cubis (unpublished)
  - EHPRO Rebuildable Kayfun 3.1(Geiss, 2016)

Scrutinizing EC literature (possible red flags)

- **Using older 2nd Gen atomizers at high power**
  - CE-types has poor quality control, huge variations in resistance, poor performance at ANY power setting (Gillman *et al, RTP, 2015*)
  - Some single- or double-bottom-coil atomizers also perform poorly at high wattage (Protank, Aerotank, Evod, all not popular now)
  - Users are favoring 3rd Gen "subohm" devices which are displaying *very* low aldehyde emissions…*because 2nd gen devices perform poorly!*

- **Lack of appropriate controls or comparisons**
  - Claim flavors are primary toxin source, but don't have complete unflavored data.
  - Too many variables
    - Different e-liquids used on different device types
  - No comparison to smoking: ECs are THR, not 100% safe
  - Unprecedented results on well-studied devices

- **Lack of understanding of EC equipment or usage**
  - Report voltages, but not wattages and/or resistance ($W = V^2/ohms$)
  - Confusion between power supply and atomizer
  - No user verification of dry puff with high aldehyde emissions
    - It takes very little aldehyde emissions to produce bad, if not intolerable, taste.
    - Users will generally not vape a device emitting high aldehydes.

Scrutinizing EC literature (possible red flags)

- **No ability to verify study**
  - Source of e-liquids not disclosed
  - Not enough information about the equipment to verify study

- **Puff protocols**
  - No real standard, but there is “normal”, so comparisons can be made intelligently with other studies
  - Usual is 4 s separated every 30 s, ~55 mL/puff (mouth volume).
  - 5 s or longer, depending on the device, may not be realistic and create decomposition and dry puff
  - Unless the point of the study is to study puff durations or a new protocol

Scrutinizing EC literature (possible red flags)

- **Must use verified and validated analytical methods.**
  - Includes aldehyde emissions and diketones
  - Derivative 2,4-DNPH HPLC (modified CORESTA #75).
  - High-resolution GC-MS with internal standards for each carbonyl compound
  - Small carbonyls are difficult to determine due to complex flavor matrix, peak overlap etc.
  - If new method is presented, must have comparative data from an independent lab using a validated method
  - Also used is Head-Space GC-MS
  - GC-FID can give false positives for diketones (overlap with other compounds)
  - Needs to have low LOD/LOQ

Citations

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