An Evaluation of Electronic Cigarette Formulations and Aerosols for HPHCs Typically Derived from Combustion

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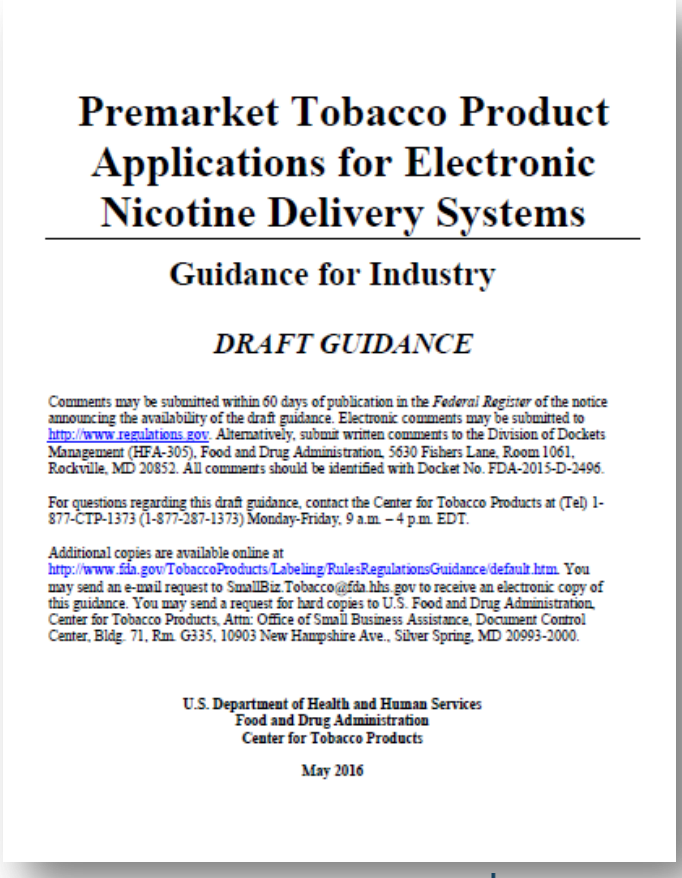
US FDA 2016 Deeming - HPHCs

"FDA recommends that you consider the following constituents for analysis in e-liquids and aerosols, as appropriate, for your product:."

"These constituents are constituents that, to FDA's current thinking, potentially could cause health hazards depending on the level, absorption, or interaction with other constituents."



Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems

Guidance for Industry

DRAFT GUIDANCE

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HPHCs in E-liquids and Aerosols [1]

Origin	Compounds
Base formulation	nicotine, glycerol, propylene glycol,
Impurities and flavors	diethylene glycol, ethylene glycol
	menthol, diacetyl, acetyl propionyl, ammonia
Nicotine related impurities	anabasine
	NNK, NNN
Leachables	cadmium, chromium, lead, nickel
Thermal degradation products	formaldehyde, acetaldehyde, acrolein, crotonaldehyde
Combustion related compounds	benzo[a]pyrene
	1-aminonaphthalene, 2-aminonaphthalene, 4-aminobiphenyl
	acrylonitrile, benzene, 1,3-butadiene, isoprene, toluene

1. Guidance for Industry, Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems, May 2016

Combustion related HPHCs

- B[a]P, aromatic amines, and VOCs are products of incomplete combustion[1-4]
- E-cigarettes have low operating temperatures relative to tobacco cigarettes (i.e. ~ 200-250 °C vs 900 °C)[2,5,6]

➢ The mechanism of aerosol formation for e-cigarettes does not involve combustion

1) McGrath, T. E., Wooten, J. B., Chan, W. G., Hajaligol, M. R., Food and Chemical Toxicology, 2007, 45: 1039-1050.
2) Baker, R. R., Bishop, L.J., Journal of Analytical and Applied Pyrolysis, 2004, 71: 223-311.
3) Fowles, J., Dybing, E., Tobacco Control. 2003,12: 424–430.
4) Piadé, J. J., Wajrock, S., Jaccard, G., Janeke, G., Food and Chemical Toxicology, 2013, 55: 329–347.
5) Geiss, O., Bianchi, I., Barrero-Moreno, J., International Journal of Hygiene and Environmental Health, 2016, 219: 268-277
6) Zhao, T., Shu, S., Guo, Q., Zhu, Y., Atmospheric Environment , 2016, 134: 61-69.

Overview

- Develop methods for the analysis of combustion related HPHCs in e-liquids and aerosols
  - B[a]P
  - Aromatic amines
  - VOCs
- Analyze commercial refill e-liquids and e-cigarette aerosols for combustion related HPHCs
- Investigate the formation and transfer of combustion related HPHCs using reference e-liquids
- All methods were fully validated
  - Specificity, linearity, accuracy, intermediate precision, LOD/ LOQ, stability

Validation Matrices (Reference E-liquids)

Sample ID	PG/Gly Ratio (%)	Water (%)	Nicotine (%)	HPHC Fortification
e-liquid 1	50/50	15	2.5	none
e-liquid 2	50/50	15	0	none
e-liquid 3	50/50	0	2.5	none
e-liquid 4	100/0	15	0	none
e-liquid 5	0/100	15	0	none
e-liquid 1 Low	50/50	15	2.5	Low
e-liquid 1 High	50/50	15	2.5	High

Fortified

Reference e-liquids were added to empty MarkTen® XL cartridges

E-liquid and E-cigarette Fortification Levels

15% water, 2.5% nicotine in a 50:50 mixture of propylene glycol and glycerin

Sample ID	Units	E-liquid Low	E-liquid High
B[a]P	ng/g	165	834
1-Aminonaphthalene (1-NA)	ng/g	16.7	66.7
2-Aminonaphthalene (2-NA)	ng/g	16.7	66.7
4-Aminobiphenyl (4-ABP)	ng/g	8.30	33.3
1,3 Butadiene	µg/g	9.49	45.7
Isoprene	µg/g	48.3	232
Acrylonitrile	µg/g	4.84	23.3
Benzene	µg/g	9.64	46.4
Toluene	µg/g	19.4	93.2

Sample Collection

- Puffing regime
  - 55 mL volume, 5 sec puff duration, and 30 sec puff interval, square wave puff
- Sample traps
  - B[a]P and aromatic amines: Cambridge filter pad
  - VOCs: Cambridge filter pad followed by a course-fritted impinger containing 20 mL of methanol (-70°C)
- Sample collection
  - 25 puffs (~ 100-150 mg of aerosol)

Sample Preparation

B[a]P (GC-MS, EI)	Aromatic Amines (GC-MS, NCI)	VOCs (GC-MS, EI)
Fortify sample with B[a]P-d12	Fortify sample with deuterated internal std.	Fortify impinger solution or e-liquid with benzene-d6, toluene-d8
Extract CFP or 0.3 g e-liquid with 2 mL water and 10 mL toluene	Extract CFP or 0.3g e-liquid with 2 mL of water and 10 ml of toluene	Dilute e-liquid in 10 mL methanol
Shake 20 minutes	Add TMA[1] and derivatize with HFBA[2]	Analyze impinger solution or diluted e-liquid
Remove the top layer for analysis	Load onto florisil SPE and elute with DCM	

1. TMA = trimethylamine
2. HFBA = Heptafluorobutyric acid

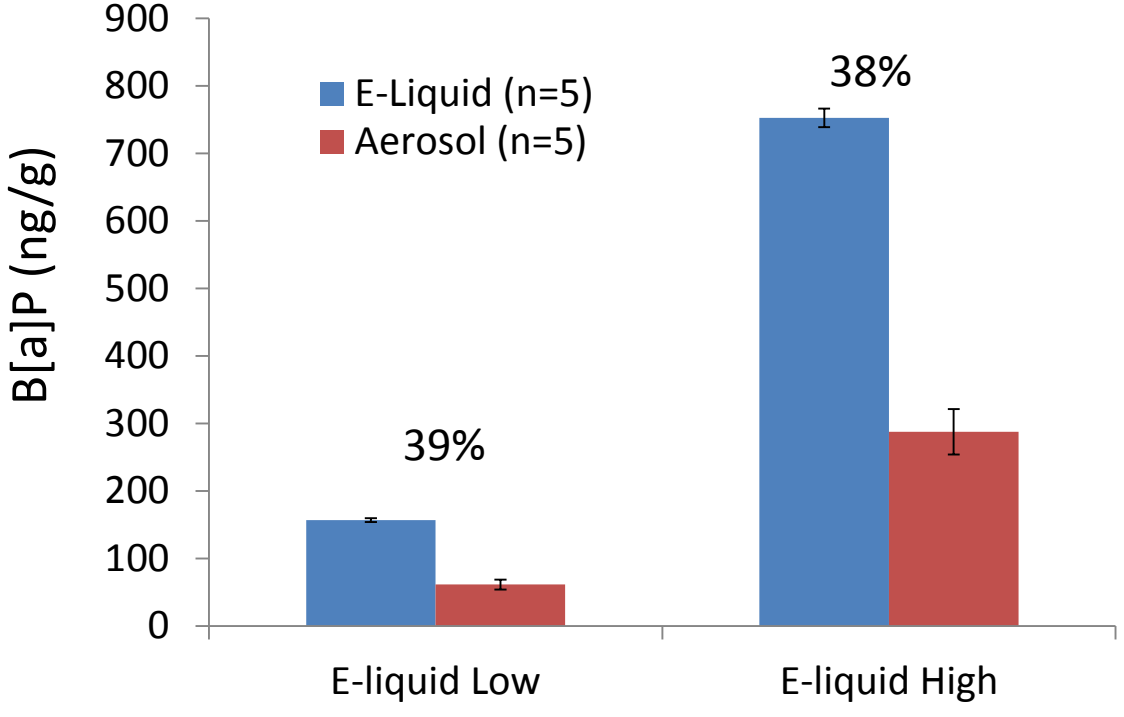
Transfer Studies

- Combustion related HPHCs were not detected in the aerosols generated from the 5 unfortified reference e-liquids
- Transfer studies were conducted using fortified reference e-liquids
  - HPHC transfer from the e-liquid to the aerosol
  - Effect of puff duration on HPHC transfer to the aerosol

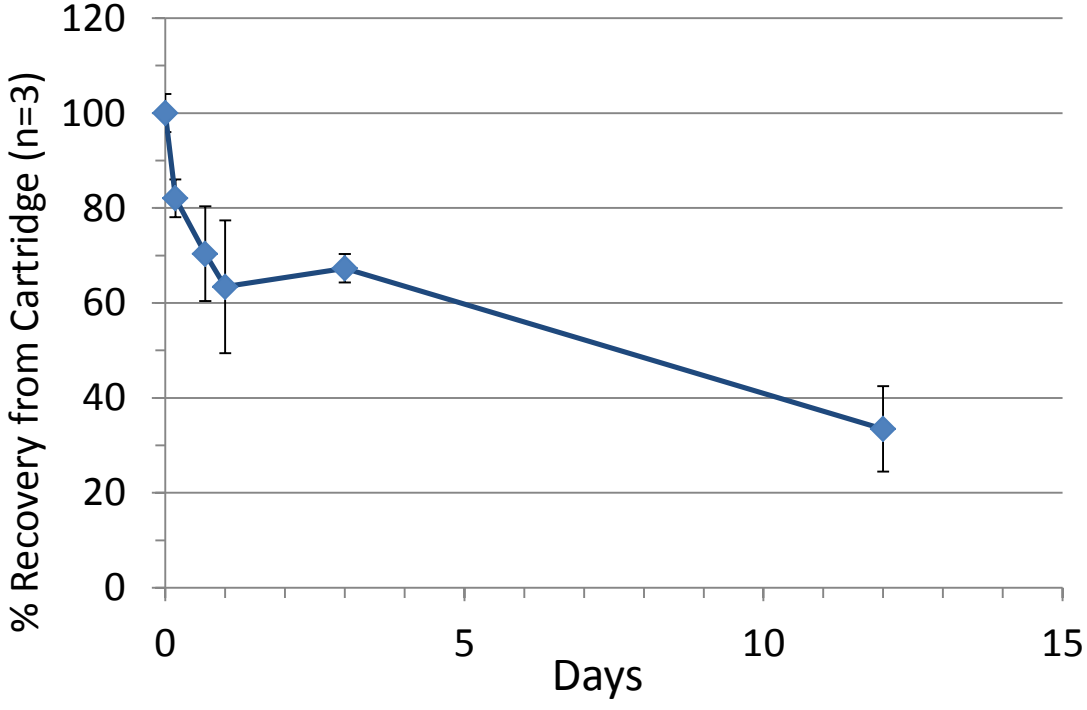
1. Reference e-liquids were added to empty MarkTen® XL cartridges

B[a]P Transfer Efficiency of Fortified E-liquid

Aerosol collected ~30 h post filling



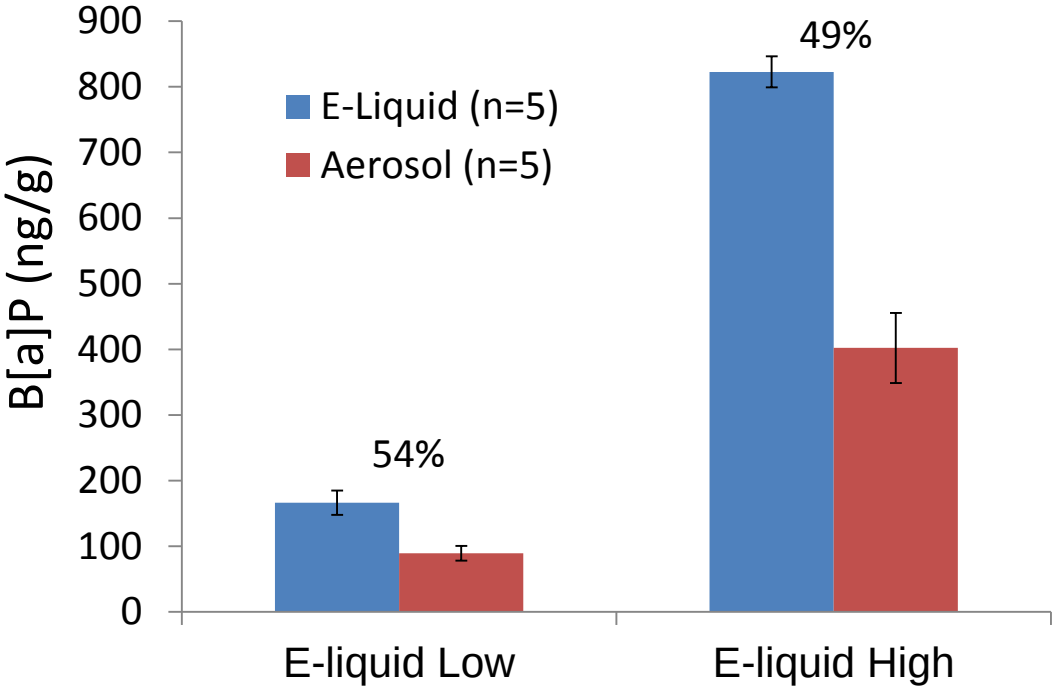
Cartridge Contact Time Study: B[a]P Fortified E-liquid



65% of B[a]P was retained by the cartridge after 12 days

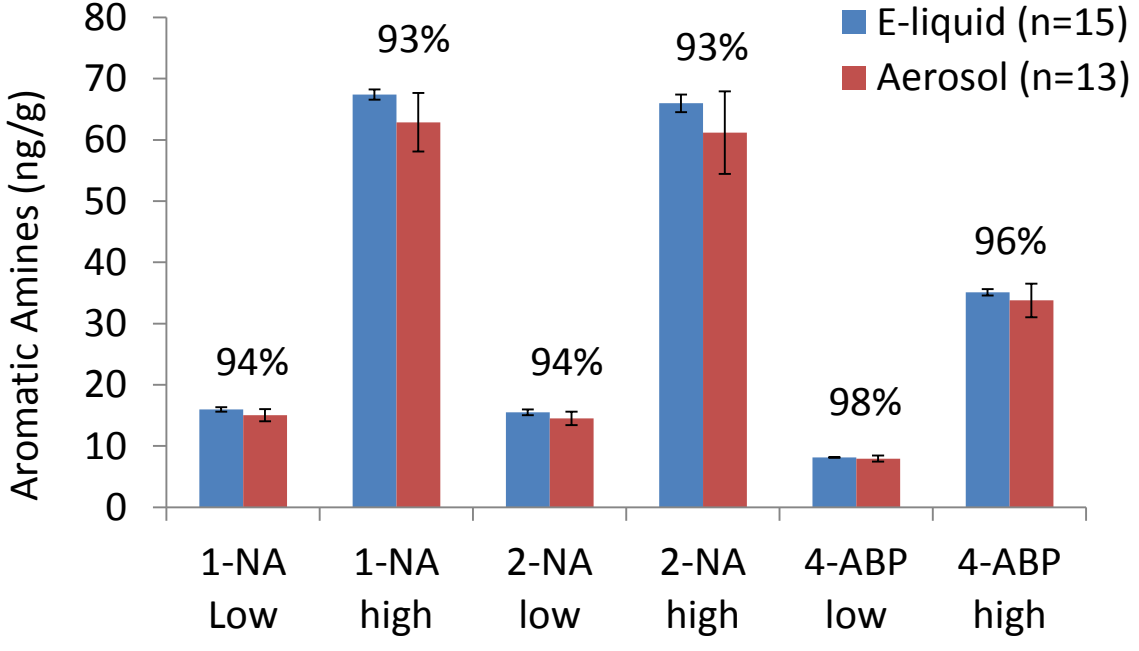
B[a]P Transfer Efficiency of Fortified E-liquid

Aerosol collected ~1 h post filling

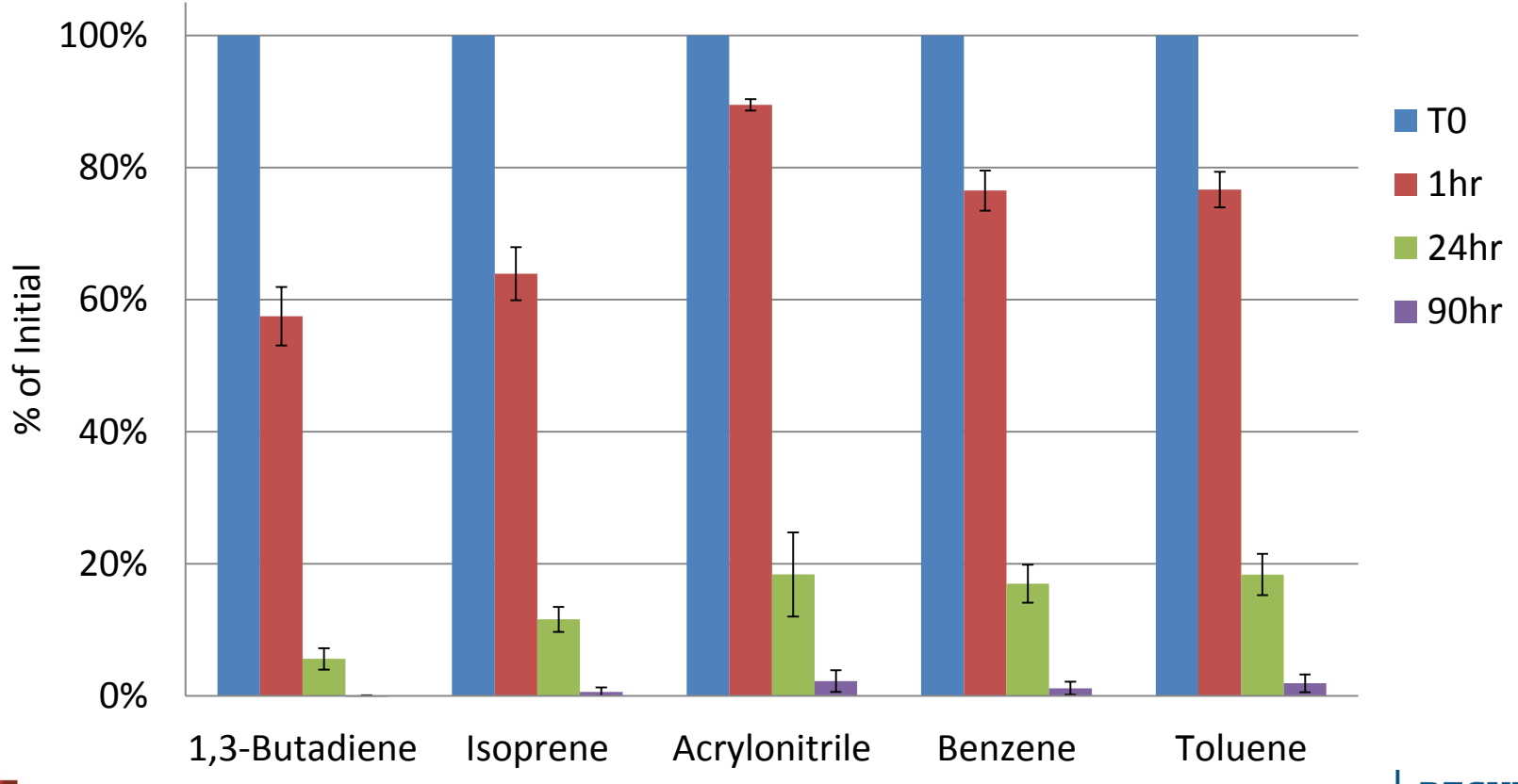


Amine Transfer Efficiency of Fortified E-liquid

Aerosol collected 13 h post filling

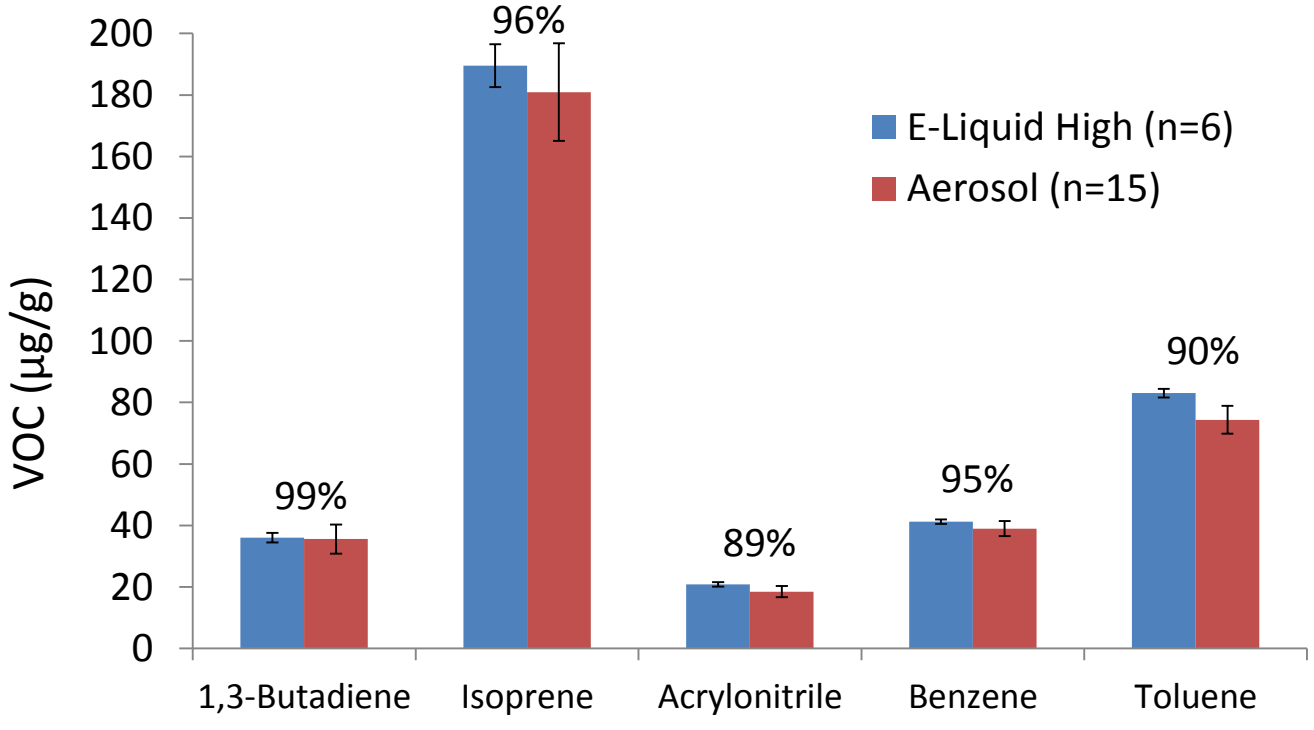


VOC Cartridge Stability of Fortified E-Liquids



VOC Transfer Efficiency of Fortified E-Liquid

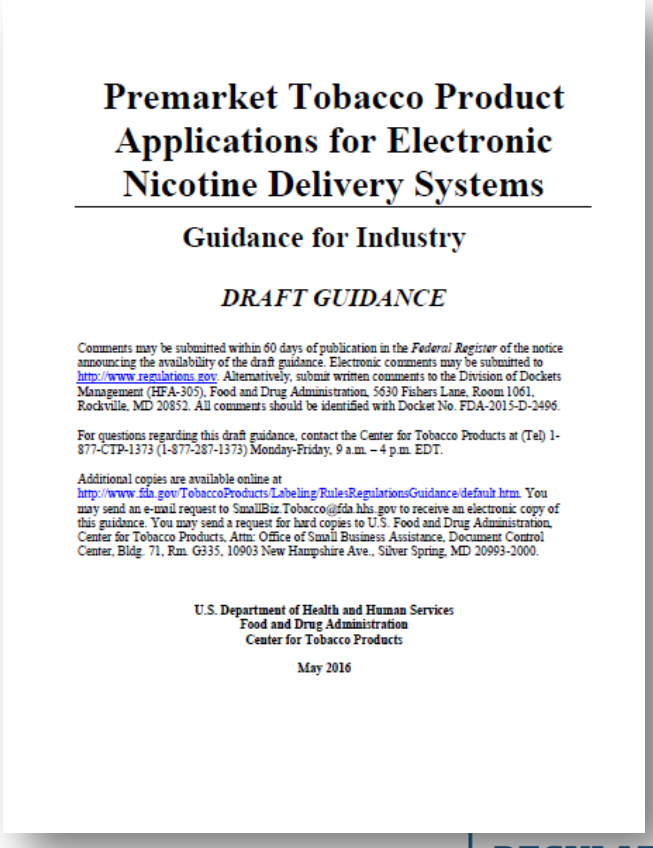
Aerosol collected 1h post filling



Aerosol Generation Guidance

"Evaluating new tobacco products under a range of conditions, including both non-intense (e.g., lower levels of exposure and lower volumes of aerosol generated) and intense (e.g., higher levels of exposure and higher volumes of aerosol generated), enables FDA to understand the likely range of delivery of emissions."

- Influence of puff duration on yield



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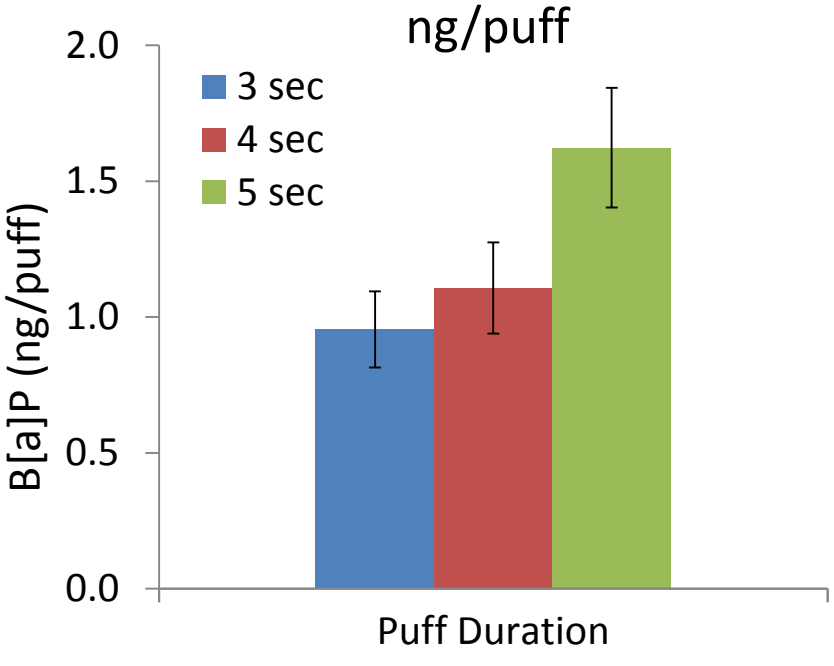
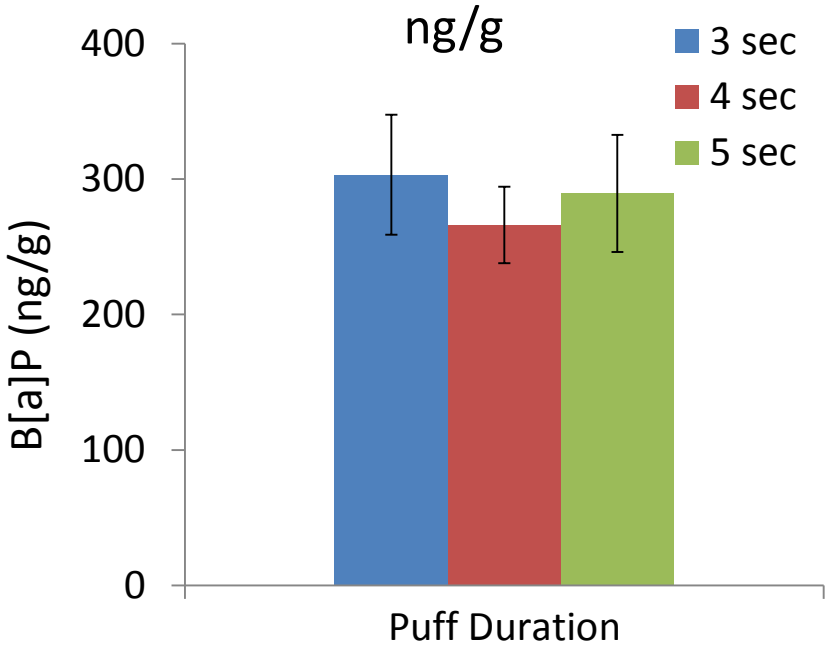
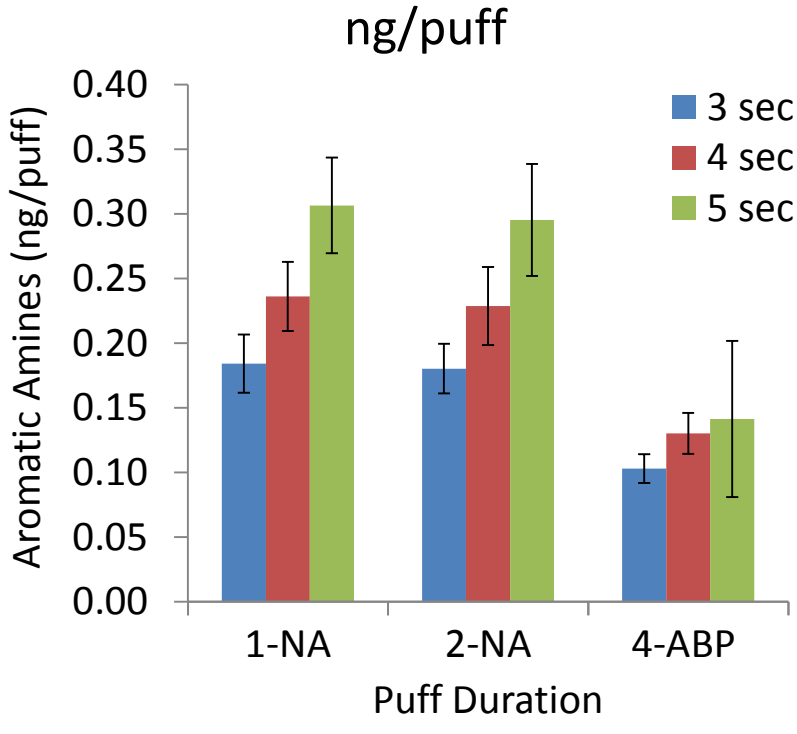
Effect of Puff Duration on B[a]P Delivery with Fortified E-Liquid[1]

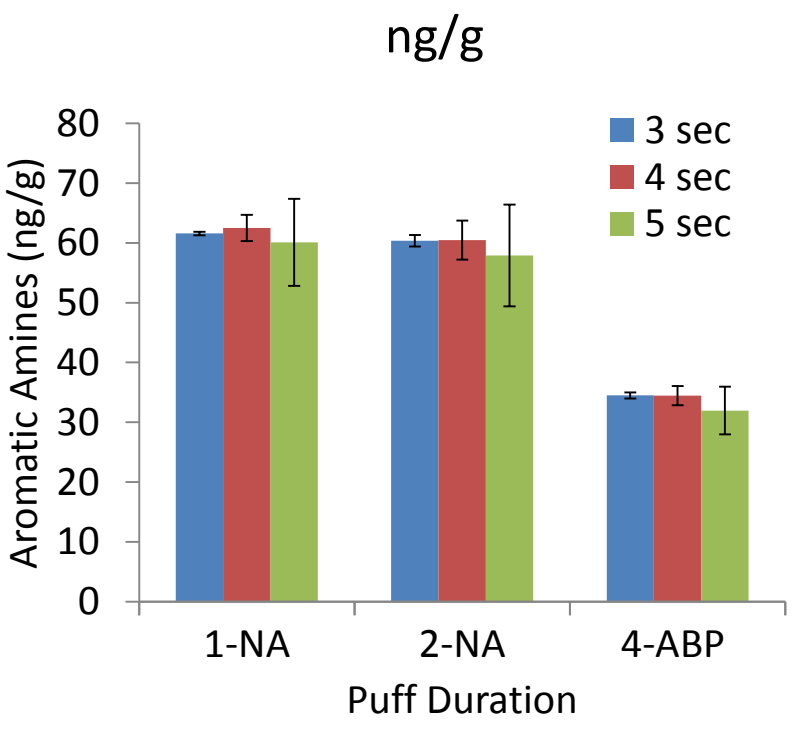




1. 55 mL volume, 30 sec puff interval, square wave puff

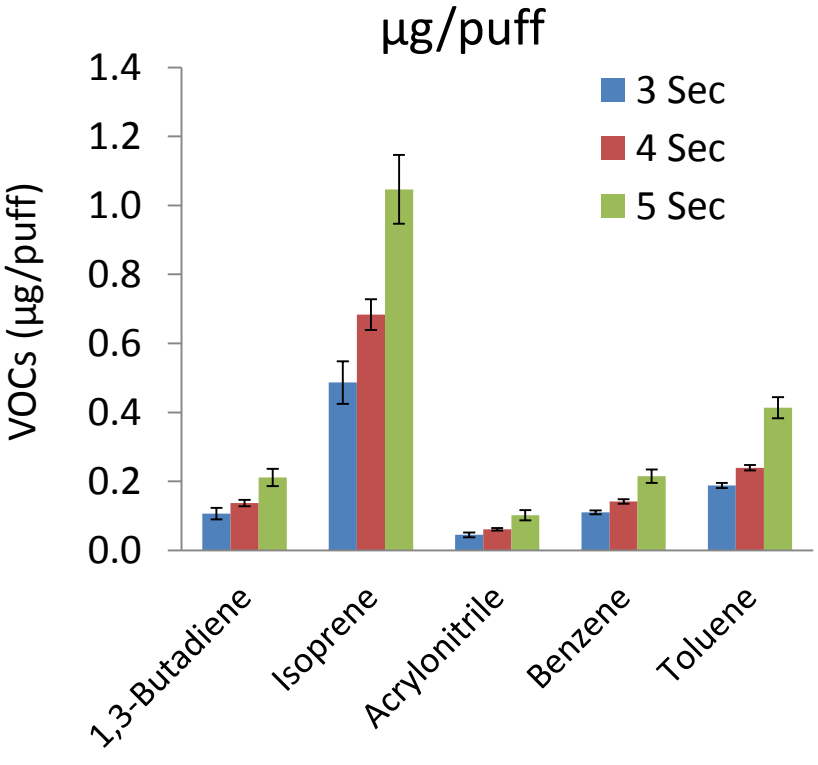
Effect of Puff Duration on Aromatic Amine Delivery with Fortified E-Liquid[1]

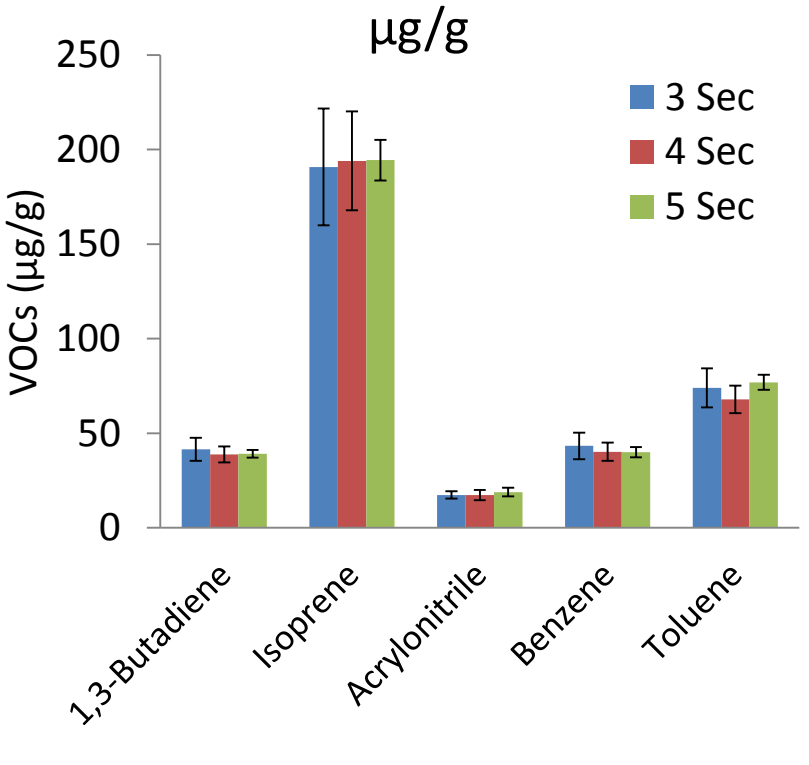




1. 55 mL volume, 30 sec puff interval, square wave puff

Effect of Puff Duration on VOC Delivery with Fortified E-Liquid[1]





1. 55 mL volume, 30 sec puff interval, square wave puff

Analysis of Commercial Products

- 13 commercial refill e-liquids covering a range of nicotine concentrations (0.6%-1.2%) and propylene glycol / glycerin ratios
  - Sweet, fruit, coffee/tea extract, tobacco

➢ None of the combustion related HPHCs were detected in any of the commercial refill e-liquids

- 6 of the top-selling commercial e-cigarettes including MarkTen® XL Classic and MarkTen® XL Menthol (Nu Mark LLC)

➢ None of the combustion related HPHCs were generated in the commercial e-cigarettes during the aerosolization process

Summary

- Developed and validated methods for the analysis of combustion related HPHCs in e-liquids and e-cigarette aerosols:
  - B[a]P,
  - 1-aminonaphthalene, 2-aminonaphthalene, 4-aminobiphenyl
  - acrylonitrile, benzene, 1,3-butadiene, isoprene, toluene
- Evaluated the possible formation and transfer of combustion related HPHCs in reference e-liquids (fortified and unfortified)
- Analyzed commercial refill e-liquids and e-cigarette aerosols for the combustion related HPHCs
- Evaluated VOC stability in e-liquids

Conclusions

- Combustion related HPHCs were not detected in commercial refill e-liquids
- Combustion related HPHCs were not formed during the aerosolization process with reference e-liquids or commercial e-cigarettes
- The combustion related HPHCs transferred to the aerosol from fortified reference e-liquids at constant concentration (per gram aerosol basis)
- The VOCs are not shelf stable in e-liquids

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