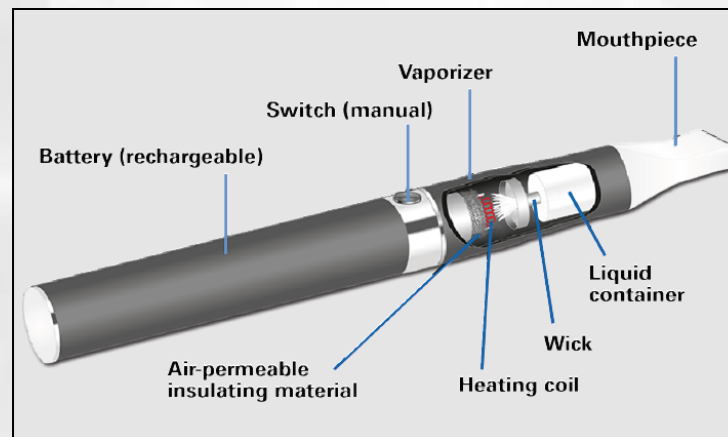


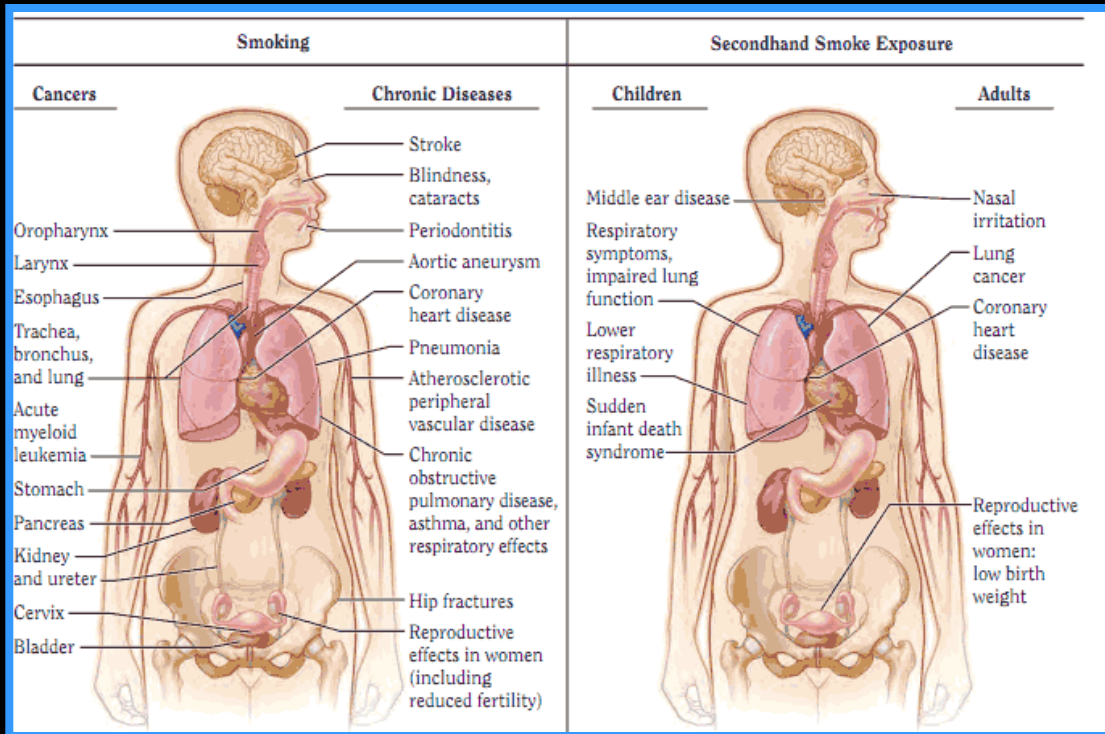
Electronic Cigarettes: How Will They Impact Human Health?



Prue Talbot
TRDRP Electronic Cigarette Webinar
10-3-2013

- Conflict of Interest: none
- Disclosures:
 - Our lab receives funding from TRDRP, training grants, and Fellowships and Internships from NIH, NSF, TRDRP, CIRM and support for a shared Stem Cell Core Facility from CIRM

Health Risks Linked to Conventional Smoking



ACTIVE SMOKER

PASSIVE SMOKER



PRENATAL EXPOSURE

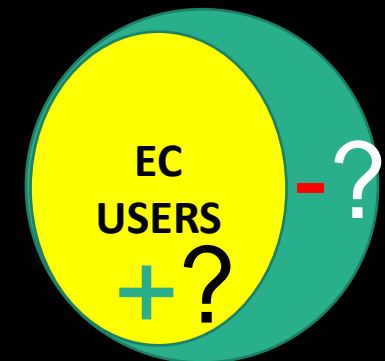
Do EC Present Health Benefits/Risks?

Are EC safer than conventional cigarettes?

Do EC reduce the risk of cancer and other adverse effects linked to conventional cigarettes?

Do any EC health effects overlap those of conventional cigarettes?

Do EC produce their own set of positive/negative health effects?



Health Effects of Electronic Cigarettes



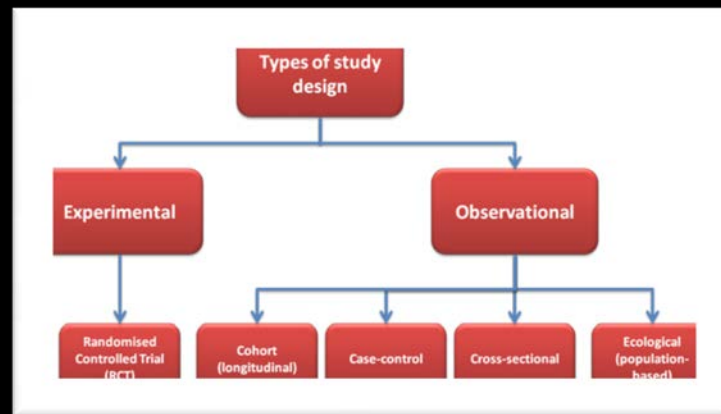
In vitro studies using cells models



Animal studies



Human studies/Clinical Trials



Epidemiological/Infodemiological Studies

In Vitro Cytotoxicity of EC Refill Fluids



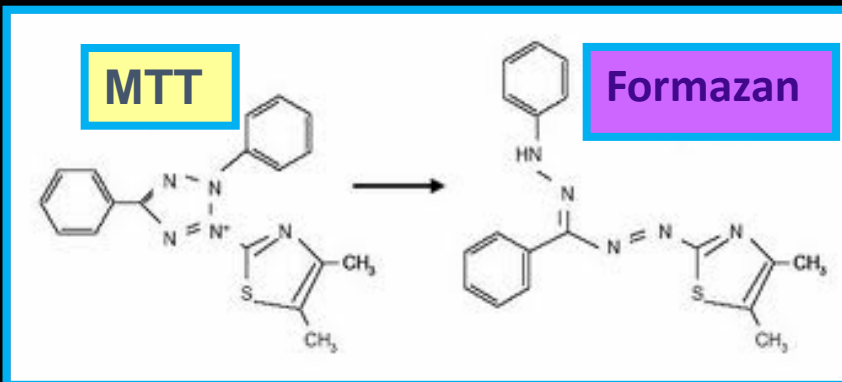
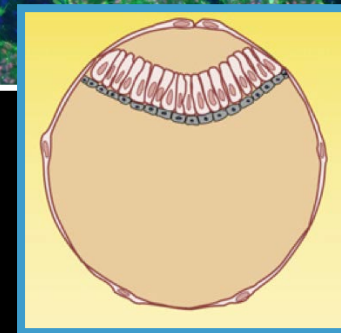
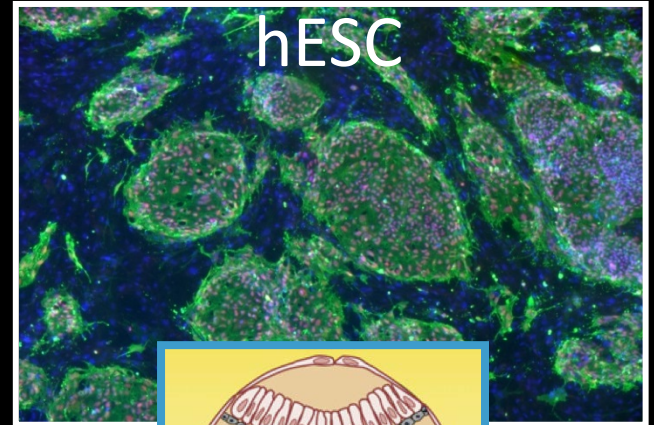
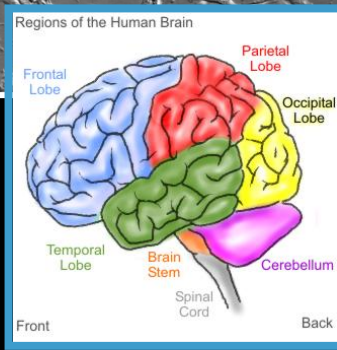
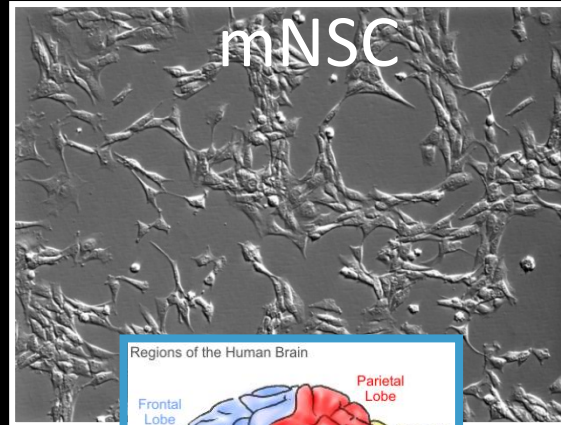
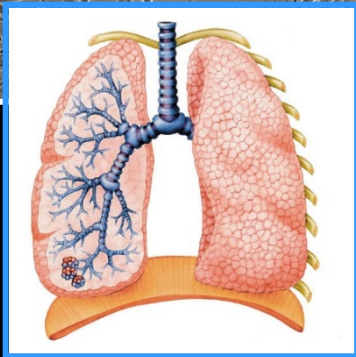
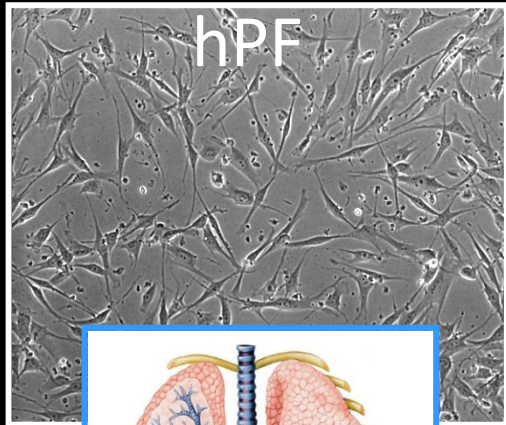
Cytotoxicity = ability of a chemical to kill cells

IC_{50} = dose that kills half the cells

- Purpose:
1. Compare relative cytotoxicity of different refill fluid products
 2. Determine if cytotoxicity varies with different cells types

Study was motivated by EC users who asked us to evaluate refill fluids that made them ill.

Strategy for Cytotoxicity Screen



- Two humectants
- Five products that made users ill
- 34 Refill fluids from 4 companies

Hierarchy of Cytotoxicity for 41 Refill Fluids

Electronic cigarette refill fluid name	Company	Nicotine (mg /ml)	hESC		mNSC		hPF	
			IC ₅₀	NOAEL	IC ₅₀	NOAEL	IC ₅₀	NOAEL
Propylene Glycol	FS-USA ¹		>1	0.3	>1	0.3	>1	>1
Vegetable Glycerin	FS-USA		>1	>1	>1	>1	>1	>1
Bubblegum	FS-USA	24mg	>1	0.3	>1	0.3	>1	>1
Butterscotch	FS-USA	0mg	>1	0.3	>1	0.1	>1	0.001
Butterscotch	FS-USA	6mg	>1	0.1	>1	0.1	>1	>1
Caramel	FS-USA	0mg	>1	0.3	>1	0.1	>1	>1
Caramel	FS-USA	6mg	>1	0.3	>1	0.3	>1	0.1
Caramel	FS-USA	6mg	>1	0.3	>1	0.3	>1	0.3
Chocolate Biscotti	FS-USA	24mg	>1	0.3	>1	0.3	>1	0.3
Caramel	Global Smoke	18mg	0.75	0.1	>1	0.3	0.41	0.01
Butterfinger	FS-USA	24mg	0.51	0.1	>1	0.3	>1	>1
Menthol Arctic	FS-USA	0mg	0.45	0.3	>1	>1	0.45	0.3
Wisconsin Frost	Red Oak	18mg	0.37	0.1	0.61	0.3	>1	>1
Domestic	Red Oak	18mg	0.37	0.1	0.31	0.1	>1	>1
JC Original	Johnson Creek	18mg	0.38	0.03	0.45	0.3	>1	>1
Coconut	FS-USA	0mg	0.35	0.1	0.68	0.3	>1	0.3
Peanut Buttercup	FS-USA	24mg	0.36	0.03	0.8	0.3	0.18	0.01
French Vanilla	Johnson Creek	18mg	0.34	0.1	0.37	0.1	0.97	0.3
Vanilla Tahity	FS-USA	0mg	0.36	0.1	0.35	0.1	0.19	0.03
Tennessee cured	Johnson Creek	18mg	0.26	0.01	0.32	0.1	>1	0.3
Tennessee cured	Red Oak	18mg	0.32	0.1	0.09	>1	>1	0.03
Island	Red Oak	18mg	0.24	0.01	0.30	0.1	>1	>1
Pure Nicotine	FS-USA	100mg	0.23	0.01	0.31	0.1	0.35	0.001
Valencia	Red Oak	18mg	0.22	0.03	0.31	0.1	>1	0.03
Tiramisu	FS-USA	0mg	0.12	0.001	0.54	0.01	1	0.1
Mint Chocolate	Johnson Creek	18mg	0.12	0.01	0.28	0.1	>1	0.1
Swiss Dark	Red Oak	18mg	0.11	0.03	0.16	0.03	0.30	0.1
Caramel	FS-USA	0mg	0.1	0.03	0.14	0.03	0.22	0.01
RY4	Global Smoke	18mg	0.09	0.03	0.09	0.03	>1	>1
Espresso	Johnson Creek	18mg	0.08	0.01	0.30	0.1	>1	0.3
Mercado	Red Oak	18mg	0.08	0.01	0.09	0.03	0.82	0.3
Simply Strawberry	Johnson Creek	18mg	0.06	0.01	0.43	0.3	>1	0.1
Arctic Menthol	Johnson Creek	18mg	0.05	0.01	0.19	0.1	>1	0.3
Butterscotch	FS-USA	0mg	0.06	0.03	0.22	0.03	0.26	0.03
Summer Peach	Johnson Creek	18mg	0.04	0.01	0.45	0.1	>1	0.3
Black Cherry	Johnson Creek	18mg	0.05	0.01	0.16	0.1	>1	0.3
JC Original	Johnson Creek	11mg	0.04	0.01	0.46	0.1	>1	>1
Chocolate Truffle	Johnson Creek	18mg	0.03	0.01	0.26	0.03	>1	>1
Tennessee cured	Johnson Creek	11mg	0.03	0.01	0.30	0.1	>1	0.001
Cinnamon Ceylon	FS-USA	0mg	0.01	0.01	0.04	0.01	0.07	0.03
Butterscotch ²	Freedom Smoke	0mg	-	-	0.58	0.3	0.26	0.03

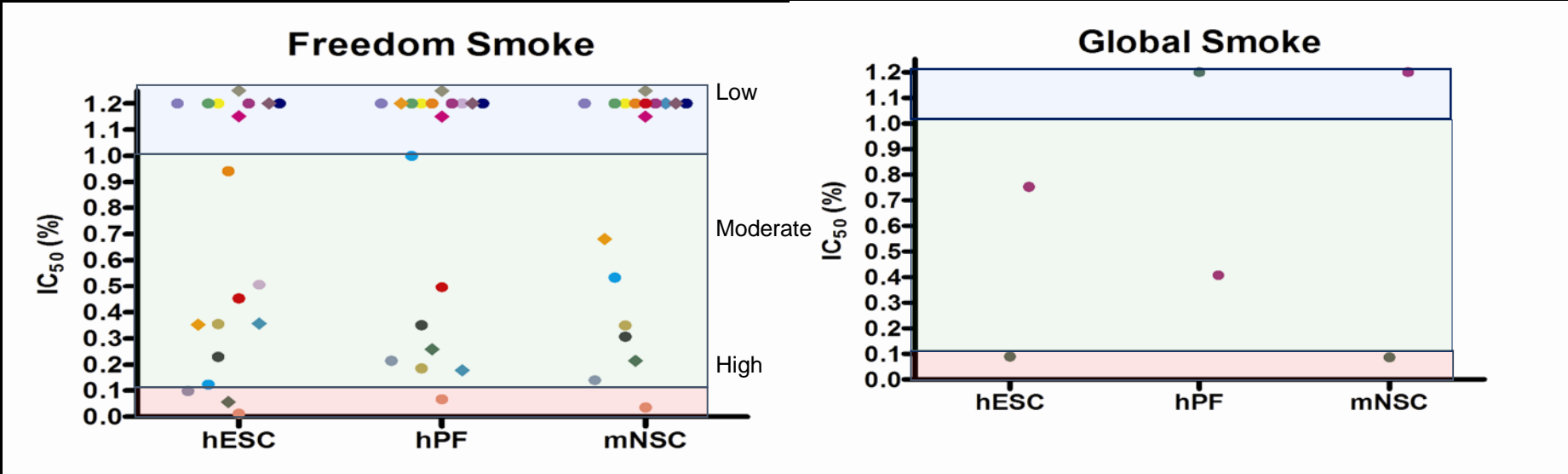
CYTOTOXICITY

Low (IC₅₀ > 1%)

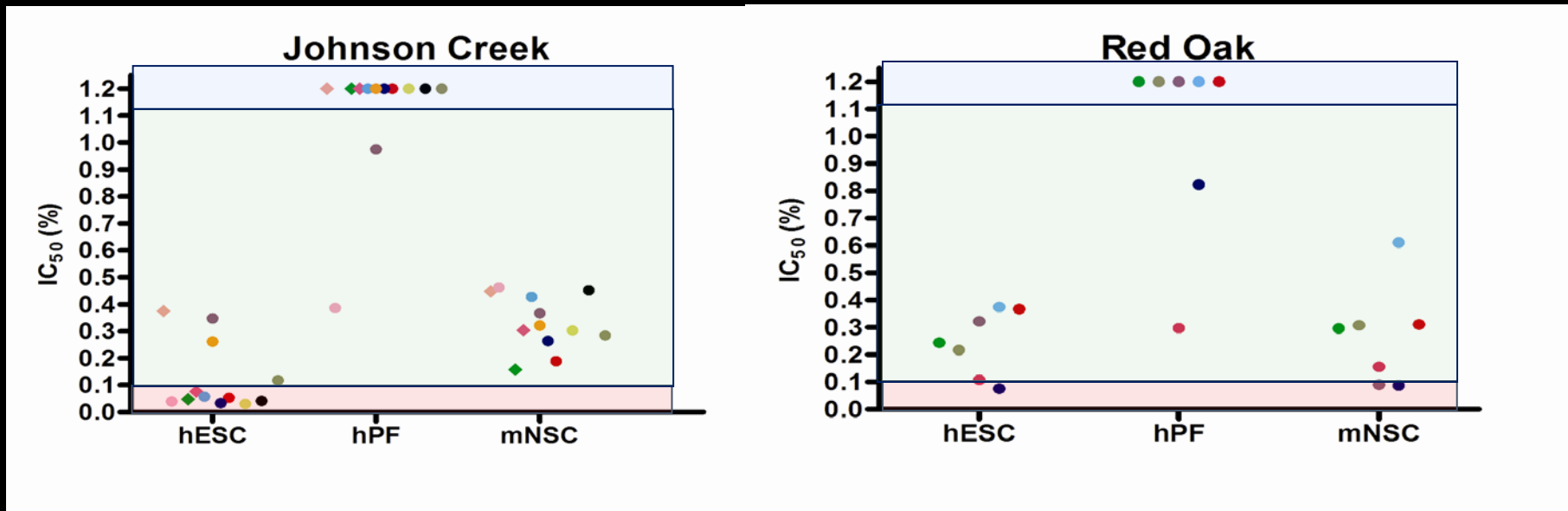
Moderate
0.1% < IC₅₀ < 1%

High (IC₅₀ < 0.1%)

Cytotoxicity of Refill Fluids from Four Companies



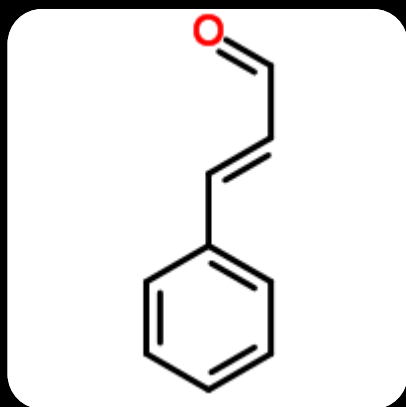
Products ranged over all three categories of cytotoxicity for all the cell types



- Most products were highly/moderately cytotoxic to stem cells and non-cytotoxic to hPF

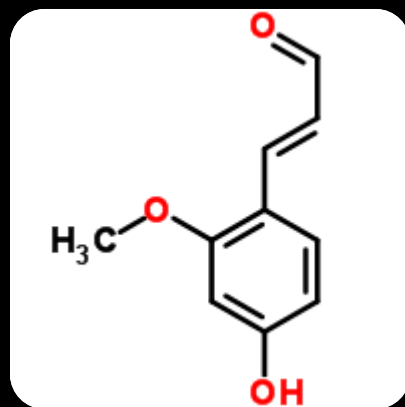
Follow-up On Cinnamon-Flavored Refill Fluids

Gas Chromatography-Mass Spectrometry was Used to Identify Chemicals in Cinnamon Ceylon



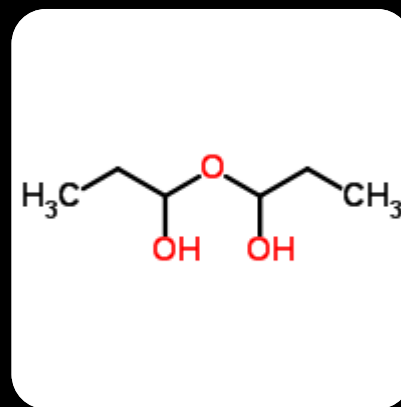
Cinnamaldehyde

HIGH



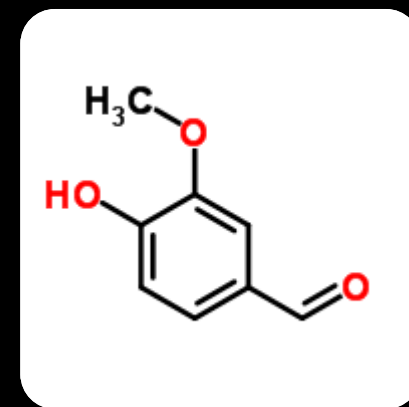
2-Methoxycinnamaldehyde

HIGH



Dipropylene glycol

LOW



Vanillin

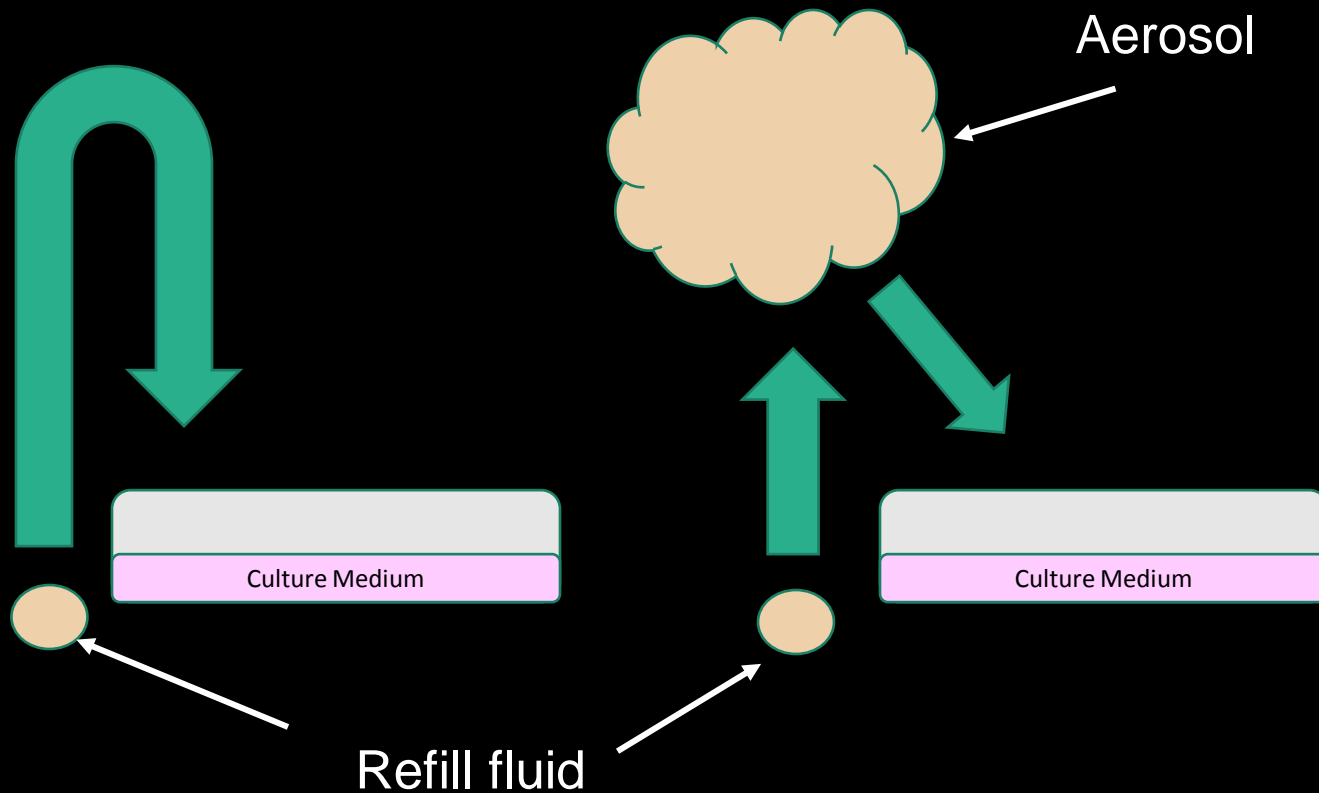
LOW

MTT Assay Was Used to Evaluate Cytotoxicity of Authentic Standards

Cytotoxicity of Refill Fluids vs Aerosol



Doses Were Prepared So Fluids and Aerosols Could Be Compared



Cytotoxicity of Aerosol vs Fluids – hPF 19 Products Compared

68%: Refill fluids and aerosols were equally cytotoxic. N = 13

11%: Refill fluid was more cytotoxic than the aerosol. N = 2

21%: Aerosol was more cytotoxic than the refill fluid. N = 4



Cytotoxicity Done on European EC Products

Romagna et al 2013 Inhalation Toxicology 25:354-361.

- Examined aerosol from 21 EC products – compared to cigarette smoke.
- Used mouse/BALB 3T3 cells –a mouse embryonic fibroblast line
- Incubated 24 hours with test dilutions of aerosol or smoke.
- Used IC₃₀ to define cytotoxicity.
- Found cigarette smoke more cytotoxic than EC aerosol.
- Found one EC aerosol (Coffee flavor) that was cytotoxic.



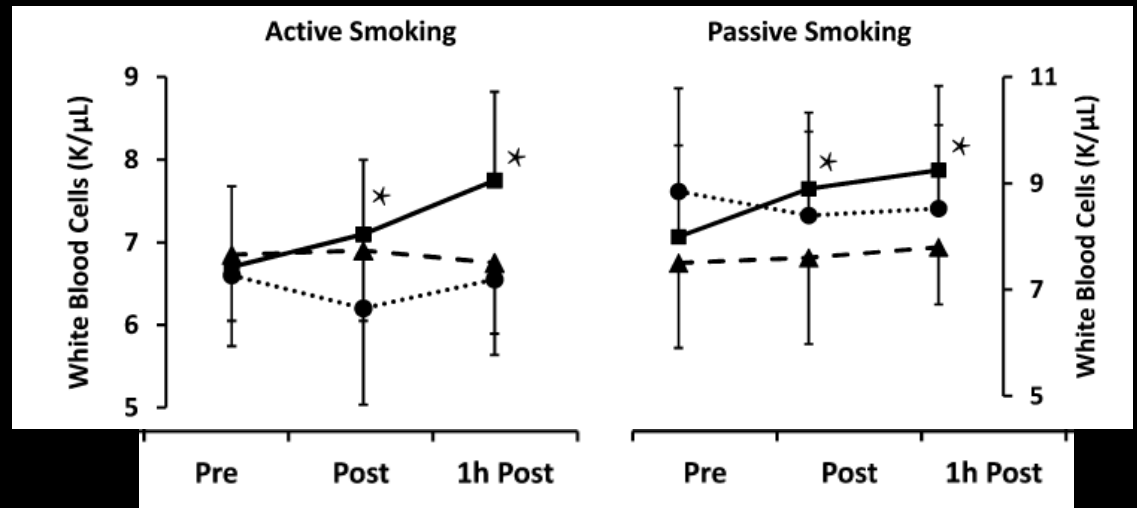
Examples of Studies Done With Human EC Users



Complete Blood Cell Count Markers Not Affected in EC Users and Those Passively Exposed

Flouris et al 2012 Food and Chemical Toxicology

- Effect of EC and CC use on use on complete blood cell count.
- Blood cell count increased in those actively or passively smoking tobacco cigarettes .
- Blood cell count was not significantly affected in those actively or passively using EC.



▲ EC smoking ■ Cigarette smoking ● Controls

Safety Assessment of EC in Smokers

Miura et al 2011 Seikatsu Eisei 55:59-64.

- 32 smokers used EC for 4 weeks (more than 150 EC puffs/day)
- No abnormal changes in:
 - Blood pressure
 - Hematological data
 - Blood chemistry



No severe adverse events were observed.

Concluded this EC may be a safe alternative to smoking.

Peering through the mist: what does the chemistry of contaminants in electronic cigarettes tell us about health risk (2013)

Burstyn, Technical Report

- Reviews recent EC peer reviewed and “grey” literature and makes predictions about compliance with occupational exposure limits.
- Concluded individual and combined exposures to contaminants in EC fall below thresholds for concern for compounds with known toxicity, including
 - volatile organic chemicals (VOCs),
 - tobacco specific nitrosamines (TSNA), polycyclic aromatic hydrocarbons (PAHs),
 - metals.
- Recommends monitoring health effects related to propylene glycol and glycerin.
 - Magnitude of the exposure is novel and at levels for concern given the lack of data on inhalation of these chemicals at levels found in EC aerosol.
- Does not consider inhalation of flavoring chemicals.



Adverse Events Reported to the FDA

Chen 2012 Nicotine & Tobacco Research



N = 47 since 2008

- **Hospitalization for:**

- pneumonia,
- congestive heart failure,
- disorientation,
- seizure,
- hypotension,
- aspiration pneumonia,
- second degree burns to face (explosion),
- chest pain and rapid heart beat,
- possible infant death secondary to choking on EC,
- loss of vision requiring surgery

N = 8

- False advertising
- Headache/migraine
- Chest pain
- Cough/sputum
- Nausea/vomiting
- Dizziness
- Sleepy/tired
- Feeling sick
- Confusion/stupor
- Sore throat
- Shortness of breath
- Abdominal pain
- Pleurisy
- Blurry vision

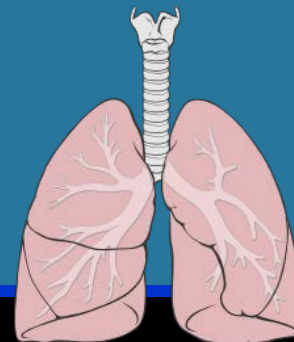
N = 39

Short Term Pulmonary Effects Using an EC

Vardavas et al 2012 Chest 141:1400-1406.

Evaluation of immediate effects of EC aerosol inhalation on airway mechanics

- 30 healthy smokers – ad lib use of EC for 5 minutes
- Controls used EC with cartridge removed
- Four parameters of lung physiology were adversely affected by 5 minutes of inhalation of EC aerosol
- Concluded short term EC use produce adverse effects similar to those seen with conventional cigarettes
- Above changes were statistically different in controls and EC users but may not be of major clinical importance



EC: Do They have a Role in Smoking Cessation?

Odum et al 2012 Journal of Pharmacy Practice 25: 611

Table 1. Undesirable Side Effects of E-Cigarettes^{13,15,16,19}

Design	Survey ^{15a}	Survey ^{16b}	Randomized Cross-over	Observational ^{19c}
n	81	3037	40	40
Duration	N/A	N/A	9 hours Per product	24 weeks
Throat				
Dry mouth and/or throat	26.2%	26.2%	N/A	8.8%
Burning/sore throat	N/A	22.1%	N/A	11.8%
Mouth and throat irritation	N/A	N/A	38%	N/A
Mouth irritation	N/A	N/A	N/A	20.6%
Throat irritation	N/A	N/A	N/A	32.4%
Dry cough	N/A	N/A	N/A	32.4%
Central nervous system				
Vertigo, headache, nausea	11.5%	N/A	N/A	N/A
Vertigo/dizziness	N/A	N/A	21%	14.7%
Headache	N/A	N/A	18%	11.8%
Nausea	N/A	N/A	29%	14.7%

Abbreviation: N/A, not applicable or not reported.

^aValues reflect percentage of comments of undesirable effects, not the percentage of patients due to the open-ended question format of the survey. The remaining values in the table reflect percentage of patients.

^bValues reported are for the 16 mg nicotine e-cigarette.

^cValues reported are at week 4.

Bullen et al 2010 Tob Control 19:98

Etter and Bullen 2011 Addiction 106: 2017

Polosa et al 2011 BMC Public Health 11: 786.

Etter 2010 BMC Public Health 10:231

Smoking Cessation Can Be Hazardous To Your Health. 2013 Shawn and Nelson Emergency Medicine 45:7-19.

- Case study of 18 month old girl who drinks about 2ml of EC refill fluid
- Rushed to emergency room with signs of nicotine poisoning
- Was given IV fluids and monitored 24 hours
- Her tachycardia and hypertension resolved
- Was discharged and appeared to be ok
- Parents were educated about safe storage of EC refill fluids.



EC Use Linked to Exogenous Lipoid Pneumonia

McCauley et al 2012 Chest 141: 1110 - 1113.

- EC user presented with exogenous lipoid pneumonia
 - Had been using EC about 7 months
- Inflammation caused by deposition of lipid in the lungs
- Chest CT showed opacities consistent with lipoid pneumonia.
- Macrophages in bronchoalveolar lavage fluid were loaded with lipid.
- Patient stopped using EC and her condition improved.
- Hypothesized condition may have been caused by inhaling EC aerosol.



FIGURE 1. Representative CT images show the "crazy paving" pattern of patchy ground glass superimposed on interlobular septal thickening. A, Bilateral upper lobes. B, Bilateral lower lobes.

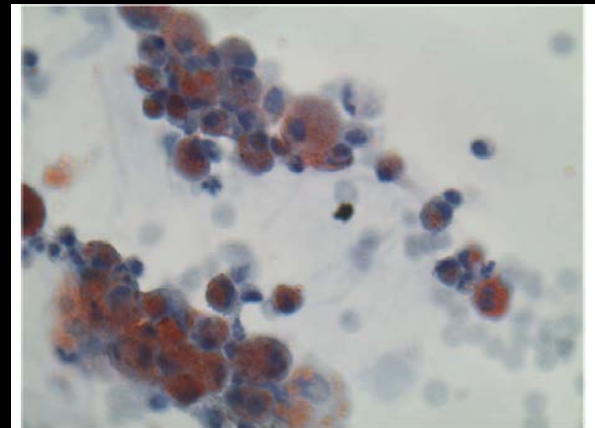
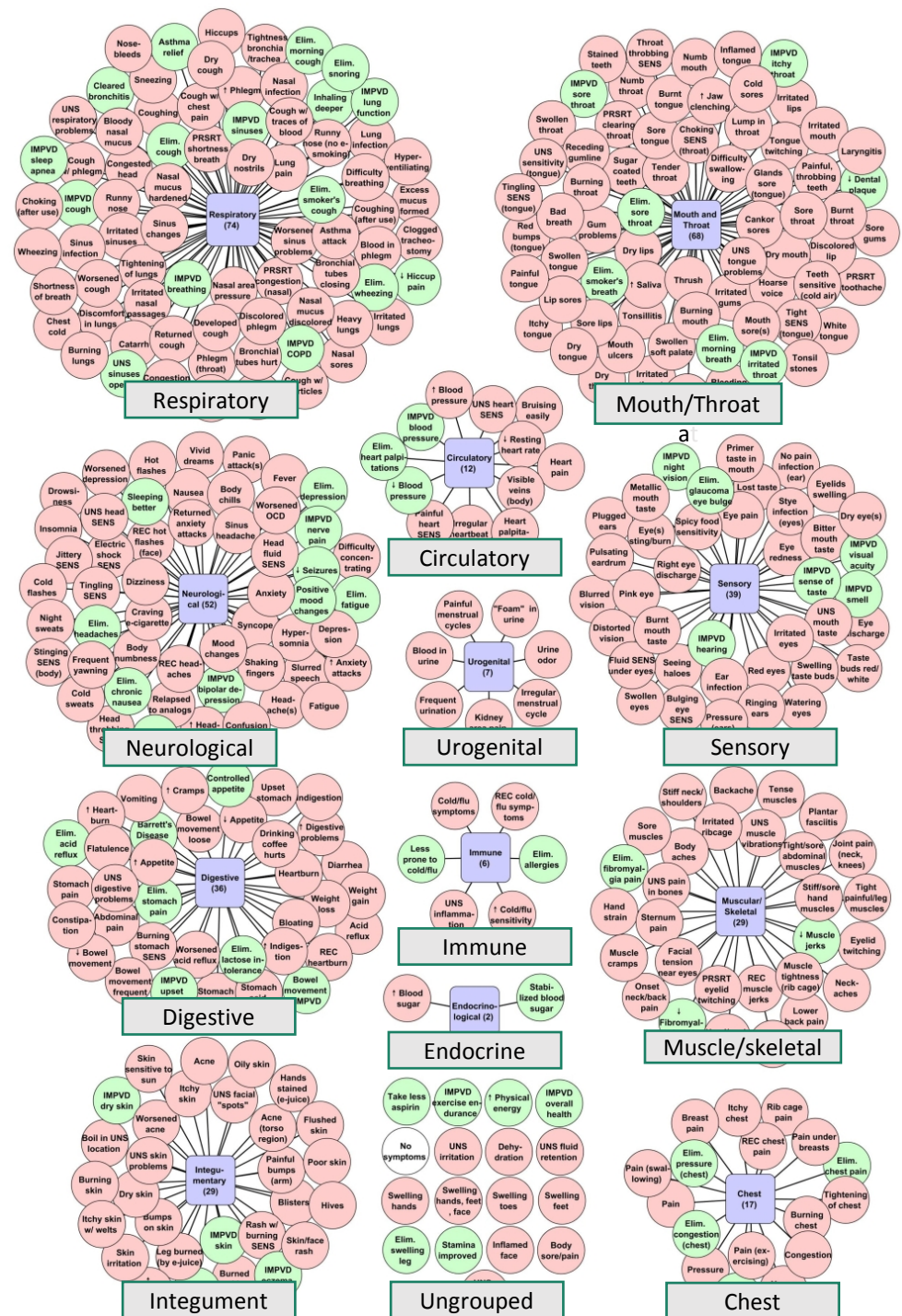
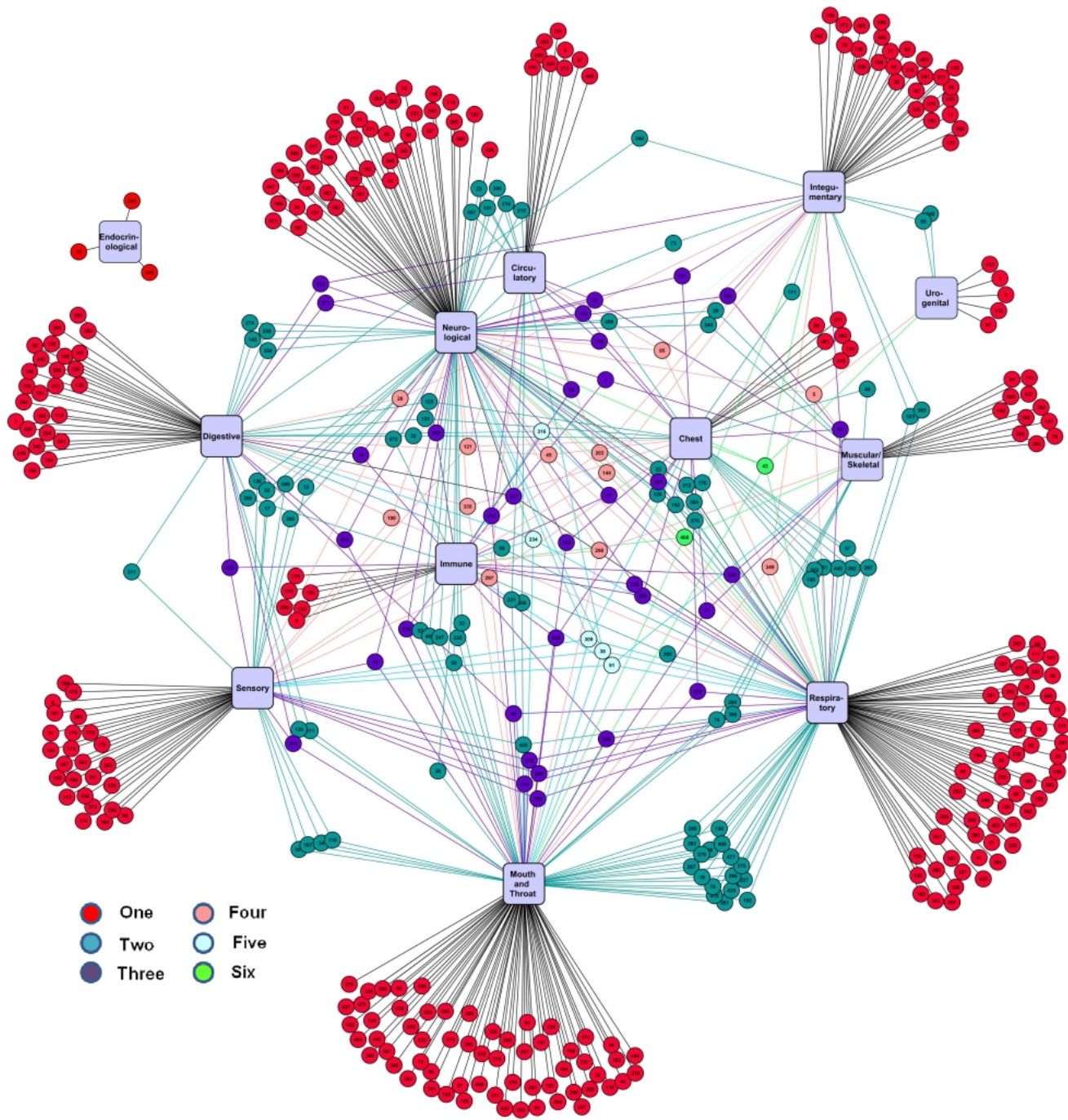


FIGURE 2. Photomicrograph of BAL sample shows lipid-laden macrophages (Oil-Red-O stain, original magnification $\times 100$).



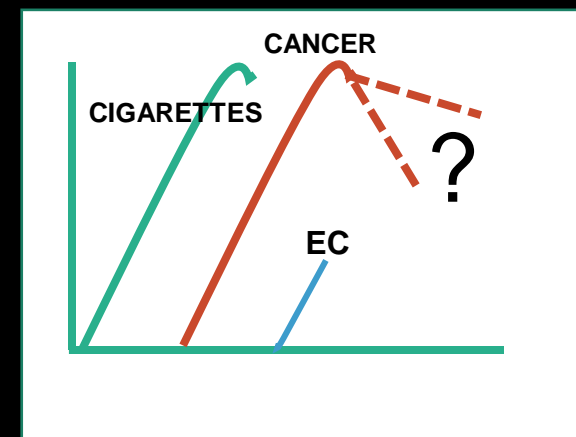
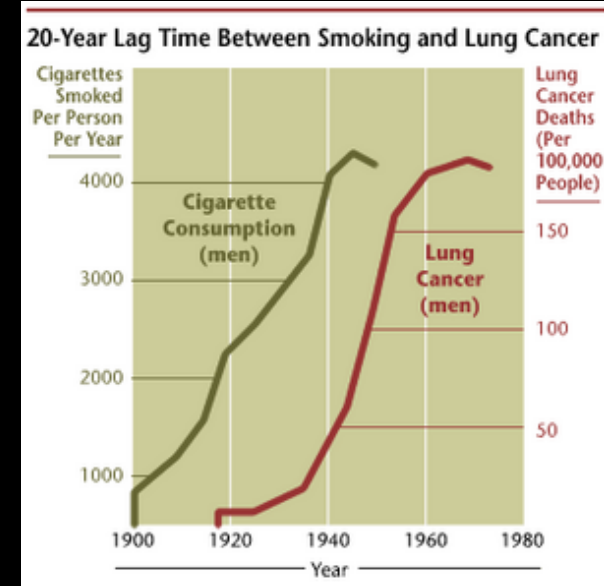
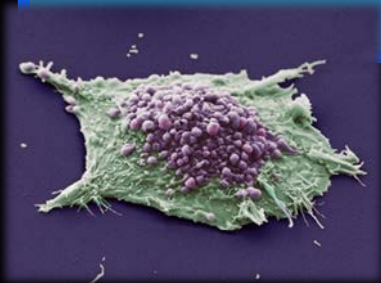
- Infodemiological Approach
- Total number of EC users = 492
- 405 different symptoms reported
- 78 positive symptoms
- 326 negative symptoms
- 12 Systems affected in EC users





Do EC Reduce the Risk of Cancer?

- This hypothesis is supported by lower levels of carcinogens (TSNA and PAHs) in EC aerosol than in cigarette smoke. (e.g. Goniewicz 2013 [Tob Control](#))
- It will take long-term studies to know if using EC reduces the risk of cancer.
- Because many individuals use EC, these studies could begin now.



Other Public Health Concerns

- EC may be a viable harm reduction product that reduces health risks in individual who use EC instead of conventional cigarettes.
- Current smokers may become addicted to EC rather than quit smoking.
- EC may be gateway products that attract young non-smokers.
- Use of EC in public places is being debated.



Children Attracted to Vaping

Children Attracted to Vaping

Overall Summary

- EC products vary in their cytotoxicity, and flavorings should be tested carefully, as they can contribute to cytotoxicity.
- Stem cells were more sensitive to refill fluids than differentiated adult cells.
- Experimental and infodemiological studies and surveys report both positive and negative health effects associated with EC use.
- It will be a number of years until we know the long-term health effects of EC, including their effect on cancer. Cancer is a major public health question. But it is not the only question.
- Much more work needs to be done on EC and their health effects.



Thanks to:

- Dr. Sabrina Lin
- Vasu Bahl
- Barbara Davis
- Rachel Behar
- Crystal Hua
- Yuhuan Wang
- Nicole Xu
- Mina Alfi
- Alex Razo
- Michael Dang
- Anna Trtchounian
- Monique Williams



Funding provided by TRDRP
UCR Deans Fellowships
Cornelius Hopper Fellowships

mNSC provided by Dr. Evan Snyder

