

# Interdisciplinary research team studies whether using e-cigarettes while pregnant causes craniofacial birth defects

22 March 2017, by Leah Small



Credit: Virginia Commonwealth University

E-cigarettes are touted as a safer alternative to tobacco cigarettes, but they could pose alarming risks to children in utero, Virginia Commonwealth University researchers have found.

According to results from a two-year study and collaboration between faculty from VCU's Biomedical Engineering and Biology departments, using e-cigarettes (also called vaping) while pregnant could cause craniofacial birth defects. These birth defects are abnormalities of the face and head that form in utero.

The study is one in a series of seven projects by research universities across the United States that look into the potential health impacts of e-cigarettes on parts of the head, face and oral cavity. Each study is funded with part of a \$2 million grant from the National Institute of Dental and Craniofacial Research, part of the National Institutes of Health.

VCU researchers aim to educate the public about the dangers of e-cigarettes and produce results that would compel tighter government regulation, said primary investigator René Olivares-Navarrete, D.D.S., Ph.D., an assistant professor in the Department of Biomedical Engineering.

"We're not going to be able to stop everyone from using e-cigarettes," Olivares-Navarrete said. "But at least we can tell them this is your choice and give strong evidence about what can happen."

Co-investigator Amanda Dickinson, Ph.D., an associate professor in the Department of Developmental Biology, said the VCU study is one of the first to investigate how certain chemical [compounds](#) found in e-cigarettes could be linked to orofacial disorders.

"There's no real study showing why vaping during pregnancy isn't safe, or when it's most dangerous during development," she said.

Olivares-Navarrete and Dickinson plan to publish the study and release numerical data on their findings this summer.

Dickinson said many smokers believe vaping isn't harmful because e-cigarettes expose the body to fewer compounds than contained in tobacco cigarettes. This does appear to make e-cigarettes less dangerous, she notes, but it's still best to stay away—those who vape inhale carcinogens, such as formaldehyde and benzene. These are some of the same chemicals found in tobacco cigarettes. There's also no long-term research about the damage vaping could cause to the body over decades of use.



Allyson Kennedy, a researcher in Amanda Dickinson's biology lab, works with frog embryos under a microscope. The embryos are used to model human pregnancies during testing. Credit: Leah Small, University Public Affairs

help standard cigarette smokers quit the habit, and vaping is strongly correlated with other tobacco use, according to the Surgeon General.

But some official opinions differ. The Royal College of Physicians, the United Kingdom equivalent to the Surgeon General, said e-cigarettes should be encouraged as a healthier substitute to standard cigarettes. Last year, the organization released a report stating e-cigarettes rarely led nonsmokers in the U.K. to take up smoking of any kind.

### Building strong evidence

Dangerous compounds are found in a variety of [e-cigarette](#) flavors, which range from bright fruit tastes to smooth crèmes. These flavors are blended with mixtures of nicotine, glycol and vegetable glycerin to form e-liquids—which fuel electronic cigarettes.

Various researchers and health regulatory organizations are in agreement with Dickinson. The Federal Food and Drug Administration banned the sale of e-cigarettes to consumers under 18 earlier last year, and now requires manufacturers to submit ingredients for approval.

A December 2016 report from the Surgeon General cautioned against the widespread use of e-cigarettes by youth, but it acknowledged e-cigarettes are less hazardous than tobacco cigarettes. The report stated research shows use of any nicotine products by minors is unsafe.

Olivares-Navarrete said one of the reasons e-cigarettes are so popular is because young people mistakenly don't associate their use with smoking.

"The part that is amazing to me about e-cigarettes is that if you ask people who use them if they are smokers, they will say no, because for them, it is not smoking," Olivares-Navarrete said.

The Centers for Disease Control found 3 million teenagers used e-cigarettes in 2015—a number 10 times greater than four years prior.

There is also no hard evidence that e-cigarettes



Suraj Kandalam, a biomedical engineering graduate student, works with a pump to infuse e-liquid vapor with saline to use during experimentation. The pump, which he created by modifying an apparatus that mimics smoking tobacco cigarettes, is meant to realistically simulate vaping. An atomizer heats up the e-liquid, turning it into vapor, which is then sucked through a syringe before it is infused with the saline. Credit: Leah Small, University Public Affairs

Determining which compounds are most harmful is a feat when there are literally thousands of flavors on the market, researchers said. Finding culprit

compounds becomes even harder when e-liquids are heated while vaping, because this results in chemical reactions that produce additional compounds.

Lab teams led by Olivares-Navarrete and Dickinson theorize certain e-liquid compounds could contribute to orofacial birth defects such as cleft palate, which is a gap in the skin of the upper lip and upper gum bones.

To gauge the risks of developing such disorders, both labs used animal models to simulate human pregnancies. The subjects were exposed to a solution created by infusing saline with e-liquid vapor.

The solution was created with an apparatus that simulates vaping. When e-cigarettes are used, e-liquids are heated and turn into vapor during each "drag." The vapor is inhaled and chemical compounds are absorbed into tissues throughout the body. The apparatus mimics this process by using an atomizer to heat the e-liquid, turning it into vapor, which is then sucked through a syringe before it is infused with the saline. The end result is the solution used in experimentation.

Suraj Kandam, a [biomedical engineering](#) graduate student in Olivares-Navarrete's lab, created the apparatus by modifying a pump used in previous studies on [tobacco cigarettes](#). The updated machine is calibrated to mimic metrics such as the timing between puffs and puff duration for e-cigarette users. Kandam said successfully modifying the machine was a way to contribute to a project with wide-reaching impacts.

"The reason I chose this project is that it's practical and easy to verbalize to the community because they already know what electronic cigarettes are. Vaping is in everyday life," Kandam said. "I thought, maybe I can prove that e-cigarettes do have an impact. All of the research is still really new. There is still a story that needs to be told."

Using the solution Kandam made, Allyson Kennedy, a postdoctoral researcher in Dickinson's lab, found incidences of cleft palates in frog embryos exposed to specific compounds in e-

cigarette flavors during a key orofacial developmental stage.

"I found certain flavors are worse [resulted in more clefts] than others. There's one called 'Nutz' and it smells like coffee. In trials with this e-liquid, we did see some clefts," Kennedy said. "It has a buttery taste that's warm, like drinking coffee. The clefts could be caused by any number of chemicals but one possibility may be diacetyl, a compound that results in the buttery taste, but is shown to be bad for humans."

If diacetyl is indeed one of the e-liquid compounds that causes clefts, it would not be its only danger when inhaled, researchers said.



E-cigarette vapor rises from a beaker while researcher Suraj Kandam demonstrates the use of a pump meant to simulate e-cigarette smoking and to create solutions used in testing. Credit: Leah Small, University Public Affairs

About a decade ago, major popcorn manufacturers stopped using the chemical after workers who inhaled diacetyl developed bronchiolitis obliterans, known as "popcorn lung." The disease causes a scarring of the lung's air sacks, which results in a thickening and narrowing of airways.

The compound is still used to flavor popcorn, caramel and other foods, but with greater safety

regulations. Diacetyl was banned from use in e-cigarettes in the U.K. last year.

Olivares-Navarrete said his lab also found that high concentrations of nicotine present in some e-liquids may not only impact facial development, but could also negatively affect pregnancy term and viability. Researchers discovered that when pregnant mice were exposed to the e-liquid, the size of their litters was reduced. This could indicate that egg fertilization is inhibited, or that some fertilized eggs don't reach full term.

"Because we are funded by the NIDCR, we mostly focus on development in the head, but it is also important to tell people [vaping] can affect the chances for you to have a baby," he said.

### **Genes, environment and compounds**

If granted additional funding, the researchers aim to embark on new studies to further document the health hazards of e-cigarettes. They plan to learn if certain genetic predispositions—combined with the environmental stimulus of vaping—result in higher incidences of craniofacial disorders.

A next step would be to map exactly which tissues in the face are affected by e-cigarette compounds during development. Researchers plan to answer practical questions, such as how long a woman who has just quit vaping should wait to conceive. They would also continue to uncover which compounds in e-liquid could contribute to craniofacial [birth defects](#).

"This is never-ending science, it's a continuum," Dickinson said. "What we are trying to do is tease apart mechanisms and get a little deeper into what is regulating the face, and thinking about gene and environment interactions."

Provided by Virginia Commonwealth University

APA citation: Interdisciplinary research team studies whether using e-cigarettes while pregnant causes craniofacial birth defects (2017, March 22) retrieved 23 October 2019 from <https://medicalxpress.com/news/2017-03-interdisciplinary-team-e-cigarettes-pregnant-craniofacial.html>

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