The Cloud Chasers

Whether it’s the now-ubiquitous e-cigarette or emerging heated tobacco products, the market for electronic nicotine delivery systems is booming. Most scientists agree that vaping is less harmful than smoking tobacco – but with limited regulation in place, how much do we know about what’s in the cloud? We meet the scientists tasked with exploring the chemical composition of these complex aerosols.

By Charlotte Barker
Analyzing Uncertainty

Hugo Destaillats is a Staff Scientist at Lawrence Berkeley National Laboratory (LBNL) Indoor Environment Group (USA), where he studies multiple aspects of indoor air quality. The group has studied tobacco smoke for many years, and as the e-cigarette market started to expand in the early 2010s, they turned their attention to the composition of vapor. The uncertainty around the effects of vaping intrigued Destaillats: “Today, everyone agrees that smoking is harmful. With vaping, the evidence is much less clear cut, with scientists and health agencies still debating the health impacts,” he says.

Instrumental in the team’s e-cigarette research was Mohamad Sleiman, now an assistant professor at SIGMA Clermont (France), who has an interest in developing analytical methods for environmental applications.

What’s in the cloud?

“We decided to study the chemical composition of vapor, to predict how it might impact on the user and those around them,” says Sleiman. The team were particularly interested in following up on previous reports of potentially toxic aldehydes found in vapor, and wanted to discover how these compounds were formed. They looked at three e-liquids and two devices to see how the technology used would impact on the composition and emission of inhaled and exhaled vapor.

The aldehydes were captured by dinitrophenylhydrazine (DNPH)-impregnated silica gel cartridges, and analyzed by HPLC with UV detection. Other volatile organic compounds were captured using sorbent tubes and analyzed by TD-GC/MS. “To gather additional information on the source of the toxicants we used headspace GC-MS – heating propylene glycol, glycerin and complete e-liquid to see if we could recreate formation of specific toxicants, and track changes in chemical profile with increasing heat,” says Sleiman.

The team found a total of 31 potentially toxic substances in the vapors they analyzed (1). “Our findings were consistent with other studies, but we made some additional observations, including two toxicants (one in vapor and one in liquid) that hadn’t been previously detected,” says Destaillats.

“One novel finding was that propylene glycol and glycerin in e-liquids can undergo thermal decomposition under certain conditions to produce the aldehyde acrolein – a powerful irritant,” adds Sleiman. Acrolein can occur at relatively high levels, depending on how the e-cigarette is used, adds Destaillats. High levels of aldehydes are sometimes attributed to unpleasant-tasting “dry puffs”, where the liquid burns rather than turning to vapor. But the researchers found that acrolein was also present under conditions mimicking routine use. Detecting aldehydes was a special challenge, says Sleiman “Acrolein is very reactive and easily oxidized, so samples had to be dealt with promptly to avoid degradation.”

The researchers noted that emissions of acrolein and other toxic compounds increased as the voltage and temperature of the e-cigarette rose, and with repeated use – presumably a result of a buildup of residue within the device. “We hope that one outcome of our research has been to provide useful information to manufacturers to help them improve the safety of their devices,” says Destaillats.

In a follow up analysis, the group carried out a simple health impact assessment for the toxicants they found in vapor, using disability-adjusted life years (2). The results suggested that while vaping is significantly less harmful than tobacco smoking, it isn’t without risks.

Spoilt for choice

Destaillats and Sleiman are particularly concerned about the “unknown unknowns” in vapor. In their study they found two compounds that hadn’t been identified before – and there could be others. “There are hundreds of e-liquid flavors out there made up of a variety of compounds; add in poor quality control and there could be impurities that no-one would think to look for,” says Sleiman.

Even “safe” compounds must be regarded with caution when they are in used in ways very different to their original purpose. “For example, the solvents used in vaping are propylene glycol and glycerin – there is a large body of evidence to show that these
“Vaping is effectively a toxicological experiment being carried out with millions of people around the world.”

compounds are safe to eat, but very little to prove that they are safe to inhale in large quantities over several years or decades,” says Destaillats. “Vaping is effectively a toxicological experiment being carried out with millions of people around the world – there may be no serious health impacts, but there may be risks that are only revealed with time.”

The e-cigarette market and associated technology is evolving rapidly, says Sleiman. “Two conventional cigarettes of the same brand will be virtually identical, but e-cigarettes and e-liquids come in countless permutations, which makes it difficult to generalize findings.” That may change as more regulation comes in, he suggests, as only companies with the resources to carry out proper quality control will remain in the industry. Either way, there will be plenty of analytical challenges for the team to explore in the years to come.

Though Sleiman has now left the LBNL group to take up a position at SIGMA Clermont, France, he and Destaillats continue to collaborate on research into vaping and other environmental applications. “As long as electronic nicotine delivery systems (ENDS) continue to evolve, we will continue to provide an unbiased analytical view,” says Destaillats.

References

E-Cigarettes Versus Heat-Not-Burn

E-cigarettes heat e-liquid (usually containing nicotine, flavorings and humectants) to vaporize it, before it condenses into a droplet cloud with a similar particle size distribution as cigarette smoke. E-cigarettes are produced by myriad manufacturers and with hundreds of flavors of e-liquid to choose from.

In heat-not-burn products, cigarette-like sticks of tobacco and humectants are heated to around 240 degrees Celsius (conventional cigarettes can reach 950 degrees Celsius), releasing nicotine and volatile flavor compounds. These devices are made by tobacco companies, and are currently only available in selected countries.
Industry Insights

Chris Wright, Head of Analytical Science at British American Tobacco (BAT) Group R&D (UK), has worked in analytical chemistry for over 30 years. Starting as a government scientist measuring dioxins in food and human tissues, he later spent 10 years at Unilever, helping to ensure the safety of the company’s food and cosmetic lines. Looking for a different analytical challenge, he joined BAT in 2008, despite raised eyebrows from some of his colleagues. “There were people who I had worked with for years who reacted angrily to the move. We all know that the tobacco industry has a checkered history when it comes to ethics and transparency, and I wasn’t blind to that. But I saw changes happening in the industry, not least a move away from conventional cigarettes and towards less harmful alternatives,” says Wright.

His misgivings were lessened when he met the R&D team at BAT and found them very frank about the dangers of tobacco smoking. “I heard countless statistics about the impact of smoking on health and mortality – there was no shying away from the inherent toxicity of tobacco,” he says.

When Wright joined BAT, he says the analytical testing in the industry was still relatively low-tech, lagging behind the prevailing standards in food and environmental analysis. So he spent three years with a small team working to improve the robustness of tobacco and cigarette smoke analysis, before being presented with a new challenge: how to characterize complex aerosols from novel ENDS. “I had always been interested in non-targeted screening of foods, including working with the International Life Sciences Institute on the application of the ‘Threshold of Toxicological Concern’ concept to food chemical residues. Suddenly, I had an opportunity to do something similar in a new field, with a potentially significant impact on public health,” he says.

Attack of the vapors

Now, Wright guides R&D on technical standards, selection of analytical techniques, strategic direction for analytical science and investment in analytical technologies. He also works closely with the company’s toxicologists to ensure that the department provides robust data for product assessments.

Analyzing aerosols from e-cigarettes or heated tobacco products poses significant challenges for both chemical and biological analyses. The analytical team seeks to answer questions from ‘How does this work?’ through to ‘What substances are formed when…?’ to ‘How safe is this?’? But, says Wright, “The biggest question facing the team right now is ‘How many substances can we detect and identify simultaneously in aerosols?’”

E-cigarette vapor is typically analyzed by GC but Wright’s team are now introducing HPLC-based techniques. “When we started out, the assumption was that all e-cigarette aerosols were vapor, which would be most easily analyzed by GC. Subsequently, we found that 90–95 percent of an e-cigarette aerosol can be collected on a glass filter pad milliseconds after it is formed – in other words, it condenses very quickly.” A few years ago the team acquired two Bruker maXis high-resolution LC-TOF instruments, which are proving a welcome addition to confirm results obtained by GC.

“We are also exploring real-time analytical tools, such as SIFT-MS (Syft), which allow instant monitoring of substances in aerosols...”
and potentially rapid or at-line chemical characterization,” says Wright. Real-time analysis would benefit the product development team in particular, giving them immediate information to make go/no go decisions during early-stage design.

On the biological side, some of the in vitro assays used in toxicology have proved difficult to apply to vaping. “The humectants used in e-cigarettes (such as propylene glycol) absorb water very well, so when added to an in vitro system, they cause dehydration and shock – obscuring some of the toxicology,” he explains. “That’s one reason why much of research so far has focused on chemical rather than biological screening, but I hope to see more sophisticated biological endpoint testing being applied to e-cigarettes soon.”

Another dimension

An ongoing collaboration with Jef Focant at the University of Liege, Belgium has brought multidimensional GC analysis into the company’s analytical armory. “I first met Jef over 20 years ago, when we were both working on dioxins. Jef went on to specialize in the emerging area of GC×GC – the only technique we thought would have the chromatographic peak capacity to separate aerosols as complex as cigarette smoke or e-cigarette vapor,” says Wright.

Initially, the project was about feasibility, and concentrated on conventional cigarettes. “There had been a few publications from other tobacco companies,
showing some great separations but lots of problems with overloading and dynamic range,” says Wright. It’s a tall order to catalog every compound in cigarette smoke, not least because of the sheer volume of data generated. So the team focused on data crunching tools that detect differences, rather than analyzing every component. “We made small changes – for example, changing the adsorbents in the cigarette filter – and looked for changes in the smoke chemical profile, which allowed us look at cause and effect on a chemical level, in bite-sized chunks,” says Wright. “Our collaboration has generated some very insightful and visually striking data to distinguish even small differences in very complex aerosol samples” (1–4).

**Big in Japan**

BAT’s e-cigarette platform is relatively stable in terms of products and acquisitions, so these days analytical activities focus on managing future commercial and regulatory pressures. Currently, the team spends most of its time on the company’s tobacco heating (also known as heat-not-burn) products: Glo. “These products are proving to be a huge commercial success, and it’s important that we ensure that they are as safe as they possibly can be,” says Wright. The products have proved especially popular in markets like Japan, where nicotine-containing e-liquids are restricted, and where cultural values of discretion and consideration for others make ENDS appealing.

“In a conventional cigarette you get combustion and a lot of pyrolysis, whereas heated products induce something akin to torrefaction of the material, releasing only the more volatile compounds as an aerosol,” says Wright. Those volatile components include nicotine and various flavor compounds, but largely exclude the combustion products that are major contributors to the toxicity of conventional cigarettes.
The vapers of the future

Currently, although EU law mandates data reporting, there is no common standard for ENDS and Wright believes there may be products on the market that carry a risk of unexpected and avoidable chemical hazards. Though BAT state that they include only compounds that have a known toxicity profile in their e-liquids, that isn’t necessarily the norm in what is a largely unregulated business in much of the world. “I would like to see ENDS become more formally regulated, to provide consistency in technical standards, harmonized methods for sample actuation, aerosol generation and physical/chemical testing,” Wright says. “This would provide direct assurance to consumers and regulators that the products that will replace cigarettes have been rigorously designed and that their long-term health impacts have been fully evaluated.”

To date, the tobacco industry has focused on comparative risk reduction, but Wright believes more can be done to characterize the residual risk. “Just because a substance appears in lower levels in vapor than in smoke doesn’t mean it isn’t a health risk. Ideally, we need to set threshold levels for each compound, so that we can concentrate our efforts on those compounds that remain above threshold. To do that, we need sensitive analytical instruments and powerful data analysis software.”

References
Lion Shahab is a psychologist, neuroscientist and epidemiologist, with a focus on tobacco control: “My interest is in the use of biomarkers as a tool to motivate smoking cessation and investigate the effects of tobacco products and products such as e-cigarettes that are thought to mitigate harms.”

“Around 2011, people started approaching our group at University College London about e-cigarettes, which were just taking off at the time,” says Shahab. Based on his previous biomarker work, he secured funding from Cancer Research UK for a study examining biomarkers related to various negative health outcomes in users of e-cigarettes compared with smokers, and those using nicotine replacement therapy, such as gum and patches (1).

A lack of evidence

Shahab says that previous studies provided only limited evidence about the harms of e-cigarettes, with some focusing on biomarkers that have only a tenuous link with long-term health consequences. “For example, people have looked at changes in the inner lining of blood vessels, and claimed that e-cigarettes cause cardiovascular disease. The problem is, you see similar changes when you drink a cup of coffee,” says Shahab. Then there were the usual problems of extrapolating results from in vitro or animal studies into humans – notably, nicotine itself is far more toxic to mice than humans.

It’s also important to note that the risk of a product is not determined solely by its inherent properties, but also by how it is used. Water is safe to drink, but a teaspoon in your lungs could kill you,
“The risk of a product is not determined solely by its inherent properties, but also by how it is used.”

says Shahab. “There was a widely reported study showing that there is hidden formaldehyde in e-cigarettes – the flaw was that the machine used to generate vapor from the product was at a setting that created “dry puffing” – something that consumers avoid at all costs due to the acrid taste,” Shahab adds (2). Shahab also points to tobacco industry studies in the 1970s showing that adding perforations into the filter lowered toxin levels. In reality, no such benefit materialized, because human smokers covered up the perforations with their fingers and smoked more intensely, in order to get the same nicotine “hit”.

As e-cigarettes have become more sophisticated, there is far more variety in how people use them in terms of temperature, choice of e-liquid, and so on, which makes it difficult to estimate how the aerosols will correlate with actual exposure, says Shahab, “For that reason, my preference is always to study humans.”

The lesser evil

In the Cancer Research UK-funded study the team focused on a panel of exposure biomarkers reliably linked with long-term...
Quantifying the Risks

Ed Stephens, a research fellow at St Andrew’s University, UK, spent a decade studying health implications of heavy metals in tobacco. When e-cigarettes became popular, he quickly saw the importance of determining the chemical composition of the vapor – and giving users a straightforward estimate of the risks. In 2017 he published a paper estimating the relative cancer risk of people who vape compared with smokers or users of heat-not-burn products. We caught up with Stephens to find out more about the study, and his work in the field.

What are the challenges in vaping research?
First, there are no internationally accepted analytical protocols or reference standards in place so no two labs do things in quite the same way - it’s effectively a free-for-all. In early 2018, the Tobacco Regulatory Science Program at the NIH plans to release a standard device and liquid formulation that should allow labs worldwide to standardize their analyses. Second, we know little about the speciation of metals in vapour, such as their valence state and molecular species, and this can be a key factor in their toxicity.

What inspired your 2017 study?
I saw that there were many papers in the literature analyzing single components of vapor for toxicity, but very few taking a more comprehensive view. I decided to apply a toxicological risk method that has been previously used in tobacco research to aggregate the impact of the carcinogens reported in published studies to date. It involves a number of simplifications, but I was able to calculate a relative cancer risk of smoking tobacco or using various alternative nicotine delivery systems. As expected, smoking tobacco carried by far the highest risk, followed by heat-not-burn, then vaping, then nicotine inhalers.

What’s next for your research?
I consider the initial estimates a starting point – I’m now working with toxicologists to address some of the simplifications in the model to create a more comprehensive assessment of disease risk, including health outcomes beyond cancer.

Reference

“The unknown
Though Shahab is confident that vaping is less harmful than smoking, the risks are hard to quantify. One problem with tobacco research is that the health effects may take
a long time to materialize. “If you look at the prevalence of smoking rates in the UK and US, you see a peak in smoking prevalence in the 1950s and 1960s, and then a peak in lung cancer deaths around 30 years later, so there’s a huge time lag between exposure and associated health consequences,” says Shahab. In addition, while some biomarkers, like NNAL (a nitrosamine metabolite) have been shown in long-term studies to have a close relationship with cancer, for others, the evidence is weaker. Other toxic compounds, like formaldehyde, have no good biomarkers to estimate exposure in humans.

“The other major problem is unknown unknowns”, says Shahab. Research into vaping is informed by earlier research on tobacco cigarettes, but the chemistry is very different.

**New technology, new risks?**

Shahab’s latest research is looking at long-term users of heat-not-burn products, like BAT’s Glo and IQOS from Phillip Morris International. “Tobacco companies are keen to promote these products, which make use of their existing tobacco supply chains, and they claim that by avoiding combustion, they reduce harms,” he says. “So far the research in this area has almost all been carried out by industry, so there is a need for independent verification.”

Shahab stresses the need for long-term studies of heated tobacco products, taking into account less than perfect use. “For example, when a stick is replaced some of the tobacco is often left stuck to the heating elements, and I suspect this could lead to the formation of carcinogens over time – but that’s something that will only become apparent in long-term studies.”

**References**
