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the Cannabis Scientist™

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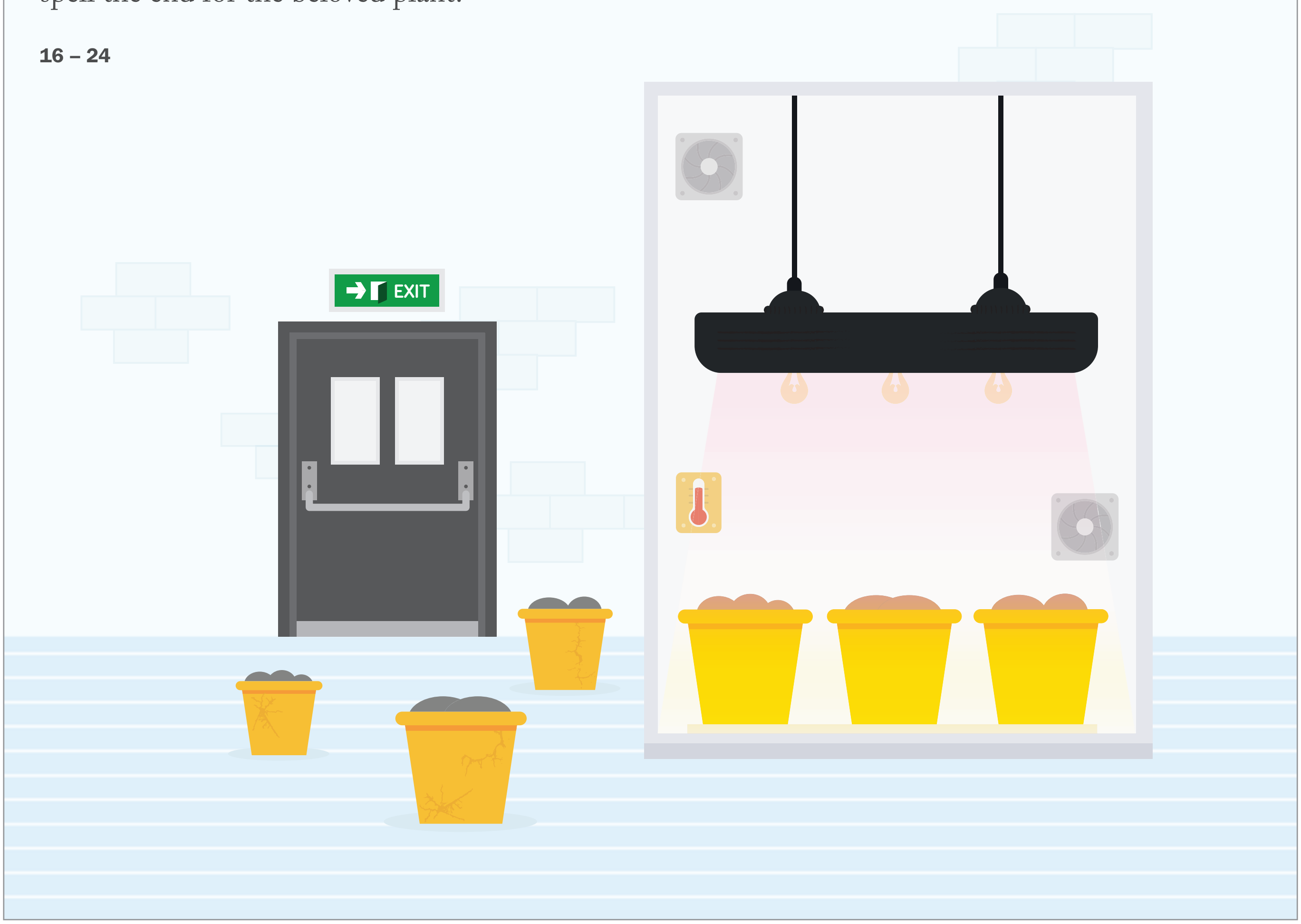


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Powering Up Cannabis Clinical Trials

At the recent International Congress on Clinical Trials in Cannabis in London, I heard a clear message from the organizers, speakers and attendees: we need more evidence. But are randomized, controlled trials the answer?

Everyone agrees that more studies need to be done on medical cannabis… But filling in the (many) knowledge gaps is slow going. Why? Some hurdles are obvious – a lack of funding and an excess of red tape make it hard for researchers in many countries to even think about conducting trials.

But even where clinical trials exist, the data from them is often contradictory, inconclusive or show only marginal improvements – a fact that seems strangely at odds with reports from patients. Should we ignore patients' experiences? Assume that they are lying to themselves or us? Or do we need to find better ways to define efficacy?

Beyond the problems of quantifying a subject experience like pain or anxiety, the mixture of cannabinoids within cannabis products can be highly variable between strains or preparations. Cannabis exerts effects not on a single target, but by weak binding to a range of receptors throughout the body – Vincenzo Di Marzo (ICB-CNR, Italy) describes the interaction as a "handshake," – a far cry from the "magic bullet" (a single molecule binding tightly to a target receptor) that is the goal of most pharmaceutical development.

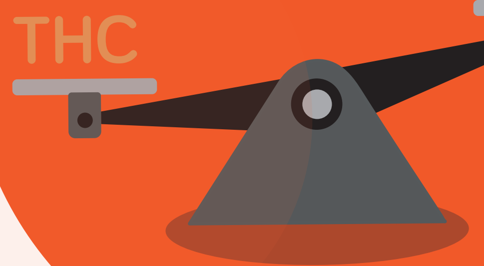
This broad action could account for some of the discrepancy between patient experience and clinical trial data. Even a small reduction in pain may make a big difference when combined with improved mood and sleep, for example. Many real-world patients don't fit into the neat boxes required in randomized, controlled clinical trials, but have a complex web of physical and emotional symptoms. We need to develop better methods to evaluate interventions for these patients – could cannabis clinical trials lead the way?

One radical approach would be to abandon traditional randomized, controlled clinical trials altogether, in favor of observational studies. In countries with centralized digital health records, the outcomes of thousands of patients can be studied across many years of treatment. And online resources for cannabis users, such as The Dosing Project and Strainprint (page 8), let patients track dosages, strains and symptoms at the click of a button.

Whichever route we choose, we need to make sure the data are robust and assessed by specialists in an array of disciplines to glean as much useful information (whether outcomes be positive or negative) as possible.

PS – Know a scientist pioneering clinical trials in cannabis? Why not nominate them for our Power List (tcs.txp.to/powerlist2020)?

Charlotte Barker
Editor



Upfront

Reporting on research, personalities, policies and partnerships that are shaping cannabis science.

We welcome information on interesting collaborations or research that has really caught your eye, in a good or bad way. Email: charlotte.barker@texerepublishing.com

Balancing Act

Does CBD really counteract the neuropsychiatric risks of THC?

Exposure to high doses of cannabis has been associated with neuropsychiatric symptoms such as paranoia, anxiety and even psychosis – and the use of high THC:low CBD strains appears to be a particular risk factor (1). Studies have shown that CBD may reduce the subjective "high" of THC in human subjects (2), and it's thought that it could also reduce the negative effects of THC. But the mechanisms behind CBD's protective effect have remained a mystery.

As is often the case with biological phenomena, researchers are looking to rodents for answers. Specifically, the hippocampus of THC-exposed rats represents a fruitful target, and one that Steve Laviolette and colleagues were determined to probe.

"We used a variety of preclinical models of THC-related side effects to measure reward sensitivity and anxiety, alongside detailed molecular analyses and measurements of neuronal activity in the dopamine system," says Laviolette. The results revealed a putative molecular mechanism behind the ability of CBD to reduce or block many of the psychotropic side effects of THC, including emotional dysregulation, vulnerability to addiction, processing problems and dopamine system disruption (3).

The team attributed these effects to CBD blocking THC-mediated hyperstimulation of the ERK 1-2 pathway in cells of the hippocampus; this pathway plays an important role in cell cycle control and cellular division, and has previously been implicated in rodent models of anxiety and depression.

The study adds to existing data showing opposing effects of THC and CBD; for example, THC dysregulates subcortical dopamine transmission, while CBD can normalize aberrant dopamine signaling (4). The researchers intend to further refine their methods, in the hope of gaining a more complete picture of how the two cannabinoids interact.

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Carbon Nanotubes for Cannabis Control

Freeing the roads of THC-intoxicated drivers, one breathalyzer test at a time

There is no reliable method for verifying cannabis intoxication at the roadside; rather, confirmation must be achieved using laborious, lab-based LC-MS methods. A portable THC detector could, therefore, streamline law enforcement efforts and protect the public. To that end, Alexander Star and colleagues have developed a handheld breathalyzer that uses carbon nanotubes to detect THC in human breath (1).

The initial model was assembled using an off-the-shelf electronics prototyping platform and 3D printed shell in a matter of weeks. "We wanted the first iteration of the prototype to be as simple and mechanically robust as possible," says Star. "To our great surprise – and delight – we quickly discovered that consumer-grade components were more than capable of meeting our needs."

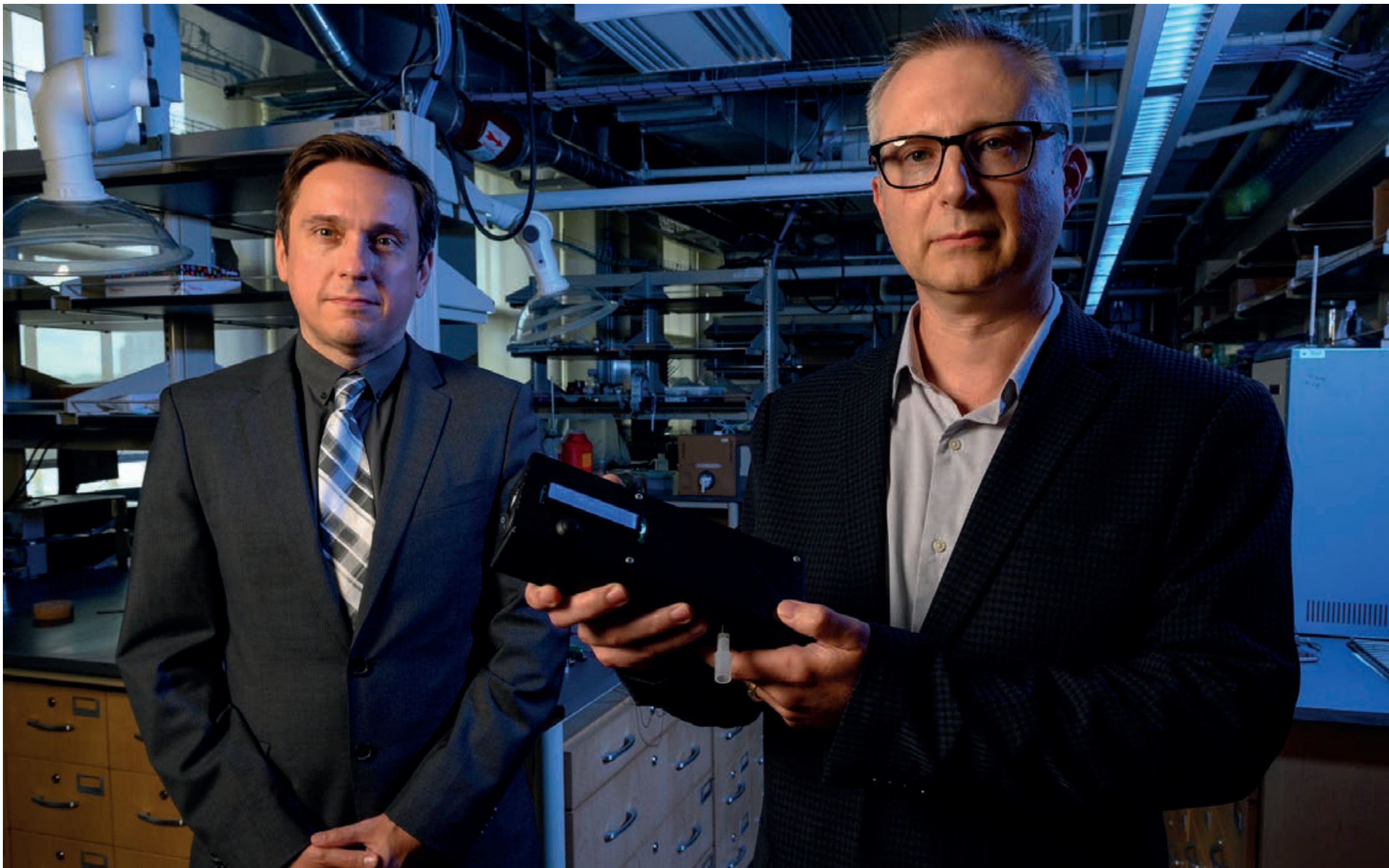
So how does the device work? As users blow into a mouthpiece, much like an alcohol breathalyzer test, their breath is directed to a chamber containing a sensor chip with semiconductor-enriched single-walled carbon nanotube chemiresistors. As THC molecules interact with the nanotubes, a measurable change in electrical resistance is generated. To enhance selectivity for THC, the team added a delay to the sensor reading, which allows more volatile components of the breath, including water, carbon dioxide, and ethanol, to desorb from the chemiresistor surface. To further increase THC selectivity, the team employed machine learning algorithms.

"We collected most of our data in the laboratory using simulated breath samples," says Star. And so now, the team is planning more extensive, real-world studies. "More prototype testing is needed to verify the efficacy of the device when detecting the small quantities of THC found in the breath of a recent smoker."

But what about the correlation between THC concentrations in human breath and driving impairment? "Building reliable, handheld THC breathalyzers will be a crucial first step," says Star.

Reference

1. *S I Hwang et al., "Tetrahydrocannabinol detection using semiconductor-enriched single-walled carbon nanotube chemiresistors", ACS Sens, 8, 2084 (2019). DOI: 10.1021/acssensors.9b00762*



Cannabis 101

With more career opportunities in the industry than ever before, academic courses are springing up to help train the cannabis scientists of tomorrow

The cannabis industry is flourishing – as a result, the demand for workers with knowledge of the plant and its applications is growing. Recognizing this, academic institutions worldwide are now offering a variety of qualifications for scientists with a range of existing experience. The University of Maryland offers North America's only MSc in Cannabis Science and Therapeutics. We sat down with the Program Director, Leah Sera, to learn more…

Why did you develop the course?

Studies across North America have revealed a considerable knowledge gap among healthcare professionals about cannabis, but, promisingly, an equally large appetite for further education. Of course, people can always pick things up "on the job", but we wanted to provide a more structured and comprehensive academic curriculum. Our objective is clear: to have a measurable impact on patient care, scientific knowledge, and public policy, by educating the next generation of cannabis scientists.

What do students learn?

We expect the majority of our students will also be working during their studies, so we designed a two-year, 30-credit part-time program. The program consists of 10 three-credit courses; four courses are required, and lay the groundwork on policy, history and culture, with a particular focus on the United States.

Students then take two courses on the science of medical cannabis. One focuses on the principles of drug action and cannabinoid pharmacology, allowing students to explore how drugs behave in the human body and how pharmacologists can exploit these processes in order to develop more effective drugs. The other is a basic course in chemistry. We don't require students to have specific scientific knowledge; instead, we try to introduce these concepts throughout the degree. The final foundational course is an introduction to patient care and the clinical uses of medical cannabis.

What about electives?

These cover everything from analytical chemistry and therapeutics to public health, advanced law, and policy. Rounding out the degree is a research methodology course that teaches students the essential skills they need to thrive in a laboratory. We've tried to make the course as holistic as possible by providing the broadest range of topics for study, so that students graduate with a background knowledge of the whole field. Our hope is that this will equip them to be the leaders of the future, able to progress the field and drive change where needed.

Jump into the Patient Pool: The Data's Lovely

How are Canadians using medical cannabis?

What?

The Canadian Medical Cannabis Experience: A 2019 Patient Retrospective Report aims to provide insights into how – and why – Canadians are using medical cannabis. The report profiles patients' symptoms, which strains and types of cannabis they used, and their self-reported treatment outcomes.

How?

Using data gathered via outcome-tracking app Strainprint, from over 800,000 anonymous patient submissions between January and December 2018 (see page 10).

Why?

Currently, there are few guidelines available to doctors or patients advising them on best practices for the use of medical cannabis. By analyzing self-reported patient outcomes, the report hopes to shed light on what dosage, strain and delivery method patients found most effective for different conditions.

Who?

The report was published by Strainprint, a Canadian data-analytics company based in Ontario, Canada.

Reference

1. *Strainprint, "The Canadian medical cannabis experience: a 2019 patient perspective" (2019). Available at: https://bit.ly/2N7udMi. Accessed October 24, 2019.*

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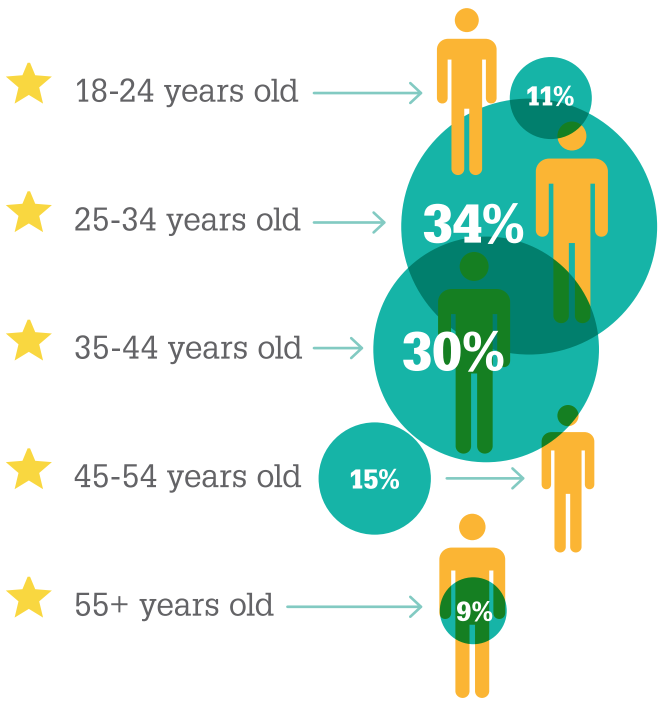
THE PATIENT PERSPECTIVE

The largest study of its kind reveals the diversity of medical cannabis users in Canada (1). We reveal some of the key findings.

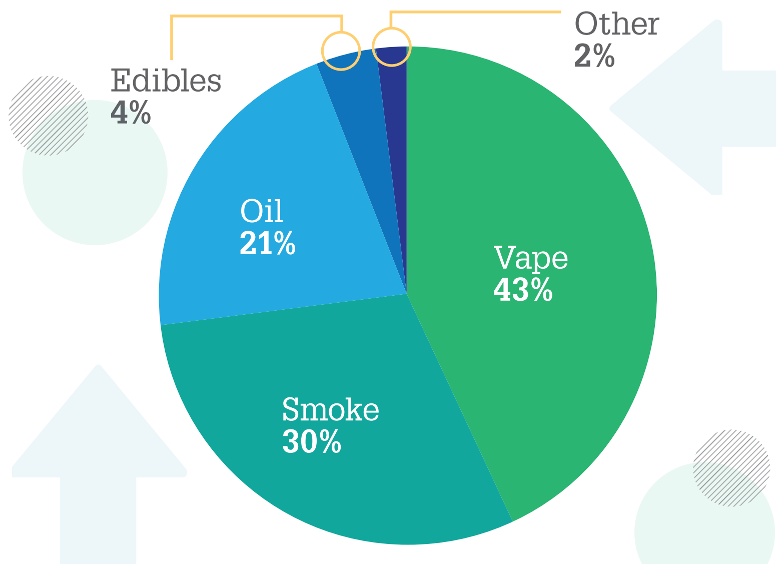
AGE OF USERS



GENDER OF USERS

53% of sessions logged by females, compared to **47%** logged by males

PREFERRED CONSUMPTION METHODS



TOP 10 SYMPTOMS

Muscle Pain **10%**

Joint Pain **10%**

Anxiety **8%**

Insomnia **5%**

Depression **5%**

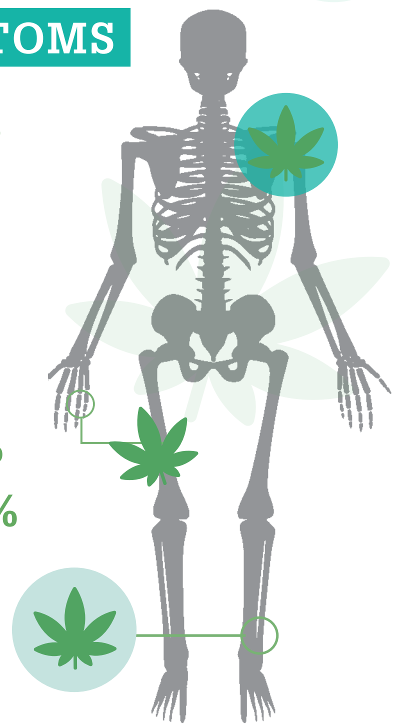
Inflammation **5%**

Joint Stiffness **5%**

Stress **4%**

Irritability **4%**

Nerve Pain **4%**



Sources: Strainprint, "The Canadian medical cannabis experience: a 2019 patient perspective" (2019). Available at: https://bit.ly/2N7udMi. Accessed October 24, 2019.

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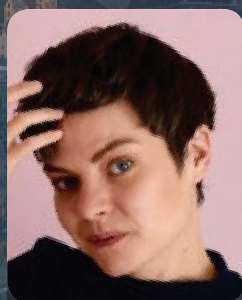

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In My View

In this opinion section, experts from across the world share a single strongly-held view or key idea.

Submissions are welcome. Articles should be short, focused, personal and passionate, and may deal with any aspect of analytical science. They can be up to 600 words in length and written in the first person.

Contact the editors at charlotte.barker@texerepublishing.com

The View from Texas

Restrictive legislation won't prevent efforts to progress cannabis research

By Kevin A. Schug, Professor, Department of Chemistry & Biochemistry, The University of Texas at Arlington, Arlington, Texas, USA.

The Lone Star State has enormous untapped potential. High-quality instrumentation and plentiful manpower at institutions such as The University of Texas at Arlington could provide the perfect platform upon which a thriving cannabis research industry might grow. The obstacle? The usual suspect: restrictive legislature. Though we are now able to work with hemp, THC-containing forms of the plant remain off limits. Even if we did pursue a license from the Drug Enforcement Administration (DEA), access to interesting strains (endemic in Oregon, Colorado and Washington) remains prohibited. Perhaps the opportunity to work with hemp without a requirement for the DEA's blessing will open new doors – at least, that's the hope; in such a rapidly growing industry, every man and his dog are looking to get a slice of the pie.

Our group remains undeterred: we've delved into cannabinoid and terpene research with aplomb, using the availability of a growing number of DEA-exempt chemical standards to our advantage. In early conversations, I was astounded by the number of people who argued that it was impossible to analyze cannabinoids using gas chromatography (GC). Liquid chromatography (LC) by contrast, is very well suited to cannabinoid analysis – holding the beacon as industry standard. Yet, GC, though certainly more technically challenging, should not be disregarded (1,2,3). The key consideration is derivatization of the cannabinoids; this preserves the carboxylic acid functionalities, which would otherwise be removed in the hot injection port of the GC instrument, and improves isomer differentiation. Having explored the utility of GC for cannabinoid analysis, we have also researched and published a comprehensive review of techniques used for the analysis of natural cannabis products (4).

This initial foray into cannabis analysis provided a much needed spark. It's become clear that high efficiency separations using LC – coupled to high-resolution mass spectrometry (MS) and tandem MS – hold real promise for cannabinoid discovery. Our group has also spent a considerable amount of time cataloging fragmentation pathways for cannabinoid ions generated by different ionization modes, which may help in both discovery efforts and the study of biosynthetic pathways in which these new cannabinoids are involved. Access to novel strains will only accelerate this progress

At the same time, we've also pioneered the use of vacuum UV spectroscopy for GC detection, which holds great potential for terpene characterization. Now, our efforts have turned towards the novel application of a commercial, on-line supercritical fluid extraction–supercritical fluid chromatography (SFE-SFC)-MS instrument. This system could be used to bridge analysis of a great range of different classes of compounds from cannabis plants – or even edibles.

It's easy to get excited by the role of analytical science in advancing the cannabis industry. Current efforts are just scratching the surface; as new therapeutic avenues for cannabinoids continue to emerge, they will need to be supported by high-efficiency, high-performance analytical methods. In short, there's plenty of (analytical) exploring left to do – even for latecomers.

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Regulation Rigmarole

Analytical scientists working on an international scale must overcome numerous challenges in cannabis research – and not just in the lab

By Kate Monks, KNAUER, Berlin, Germany.

This is an exciting time to be working with cannabis. We are in the midst of a global legislative shift, therapeutic breakthrough and economic boom. These volatile paradigm shifts are not without their challenges for those of us in the lab establishing tools for quality control, or those wanting to bring cannabis-containing products to the market.

Regulatory guidelines for cannabis and cannabinoids are non-harmonized within Europe, let alone over the world. This leads to different statuses in different countries with no common understanding among regulating bodies and their inspectors. The complexity is then further increased by the tendency for requirements to differ per the intended use: research, food stuff, medicinal and cosmetics, to name but a few. What's more, due to the narcotic nature of some cannabinoids contained within the plant, regulation constrains are further elevated. This sharpens the need for thorough analytical testing of product quality.

We love a challenge in our lab. So, when customer requests for high-pressure liquid chromatography (HPLC) analytical and purification solutions started to surge at the beginning of 2018, we decided to apply for a narcotics license for our lab to start gaining regulatory and chromatographic experience in this area firsthand.

We were aware that the process would be lengthy - we are, after all, located in Germany - but were still struck by the amount of bureaucratic adeptness we would need to bring to the lab bench. The application process handled by the Federal Opium Agency (FOA) took over nine months, from internal project kick-off to holding the permit in our hands. We started off by setting out carefully deliberated and detailed project plans,

"These volatile paradigm shifts are not without their challenges for those of us in the lab."

filled in numerous forms, underwent personal background reliability checks by the Federal Central Criminal Register, set up a secure storage including a specially designed safe, laid out process descriptions, designed forms and standard operation procedures, defined authorities and then waited… And waited… And waited. The application folder was pretty thick by the time we had everything together. I was half expecting for the authorities to ask for a blood test.

For us, as an ISO 9001 and ISO 14001 certified company, with a diligent team in the lab and well-structured system for the management of hazardous substances, it was reasonably easy to integrate requirements of the narcotics law into our everyday working. I could imagine this could be trickier for those labs without an established quality management system. However, I would recommend not to be deterred; in the end, there is additional preparation and more paperwork, but the projects are even better planned and therefore run smoothly.

Since receiving the first license, we have filed for and been granted four extensions from the FOA. The first amendment was to correct a mistake in the first application (oops), and the subsequent changes were to increase the licensed amounts to enable us to scale up to a preparative scale. The application for amendment went far more smoothly and swiftly than the first application (weeks rather than months), and - as our new friends at the FOA have unofficially hinted – further extensions to the permit should be unproblematic. That is unless or until we apply to be licensed for far larger quantities and therefore move into the next safety category. Then we will need to sharpen our pencils and adapt the management system for more forms and further safety measures.

Though the effort involved in setting up a compliant working environment for cannabis analysis and purification has been challenging, we have gained valuable insight and learned lessons which help us to assist our fellow scientists and future HPLC customers. They will in turn contribute to future cannabis and cannabinoid research, advancing the field of medicine and product quality, and increasing our understanding of this alluring herb, filling the many knowledge gaps in biology, pharmacology, epidemiology and clinical efficacy – a tasty reward all round.

Vape Flavor Regulation: a Sour Taste in the Mouth?

Regulating cannabis vape flavors may have negative consequences for wider cannabis research... Unless industry acts quickly

Brad J. Douglass, The Werc Shop, Los Angeles, USA.

The term "entourage effects" was originally introduced to describe the combined action of molecules produced by the human body on the endocannabinoid system. These effects were striking in that they influenced the binding and activity of traditional endocannabinoids, such as 2-arachidonoyl-glycerol, without binding to endocannabinoid receptors themselves (1).

More recently, the entourage effect has been used to describe similarly indirect effects elicited by molecules produced outside of the human body — most notably by the phytocannabinoids and terpenes of cannabis. In fact, recognition of such effects paved the way for the first medical cannabis systems when it became clear that FDA-approved dronabinol (purified delta-9 THC) did not provide the same benefits as the whole cannabis plant across various indications.

So, how is this relevant to vaping? As cannabis extraction has evolved in laboratories, more sophisticated refinement methods have enabled us to obtain formulations that better mirror those present in the plant. The guiding principle has become: "remain true to the original plant composition, rather than simply being whole-plant." For cannabis vapor products, this involves formulating with terpenes and terpenoids. But, as any cannabis aficionado can tell you, terpenoids impact the characteristic flavor and aroma of cannabis, and may also exert entourage effects. Accordingly, many terpenes are regulated as flavor additives for food – and for e-cigarettes, too.

Flavored nicotine e-cigarettes have been around for about a decade, but the variety of flavors available has skyrocketed in the last few years – as have concerns about the safety of inhaling flavor compounds (2). Apart from the few substances used as excipients in inhaled pharmaceutical products, there is no such designation as generally recognized as safe (GRAS) for inhalation. It is true, however, that almost all of the flavor additives used in tobacco and nicotine products are GRAS for food use, which highlights an important point about GRAS designations – they concern how a compound is consumed, rather than the compound itself. Thus, there has never been a mandate (or effort) to evaluate whether flavor compounds are GRAS for inhalation.

In the absence of safety data for inhaled flavor compounds, some stakeholders have proposed state-level legislation to prohibit all flavored vapor products – including cannabis terpenes. This state-level legislation is mainly targeted at nicotine vapor products, but cannabis vapor products and entourage effects may also become a casualty. The cannabis industry must take action to avoid this.

In doing so, we could take inspiration from The Flavor and Extract Manufacturers Association of the United States (FEMA), who pioneered the FEMA GRAS program to assess the safety of food flavor ingredients over a half century ago. Before that, safety data for thousands of flavor compounds was practically non-existent, and the US Food and Drug Administration (FDA) did not have the resources to evaluate – there was a real risk of food flavor ingredients being prohibited.

To prevent this fate, the FEMA GRAS program began with a survey of flavor ingredients in use at the time. An Expert Panel was then established in 1960 to conduct evaluations of safety and structural relationships of the identified flavor ingredients; this panel introduced several techniques now considered standard in this field (3). What's more, though FEMA provides financial support for the Expert Panel, it operates as an independent body that follows strict conflict-of-interest procedures.

The cannabis industry should emulate this model. An independent panel of cannabis experts could contribute to the acquisition of inhalation safety information, while a self-regulating body akin to FEMA could promote data-centric, defensible standards regarding what is safe and what is not. Such a Cannabis Flavor & Entourage Association (CFEA) should, like FEMA, begin by producing an inventory of the naturally occurring compounds found in cannabis – the first step towards evaluating the flavor compounds acceptable for use.

In short, a cannabis-specific approach to flavor additives is required. Industry must engage in self-regulation to establish standards and address the potential hazards of inhaled, aerosolized constituents to succeed in ensuring that entourage effects are not removed completely from manufactured cannabis products, including vapors and oils.

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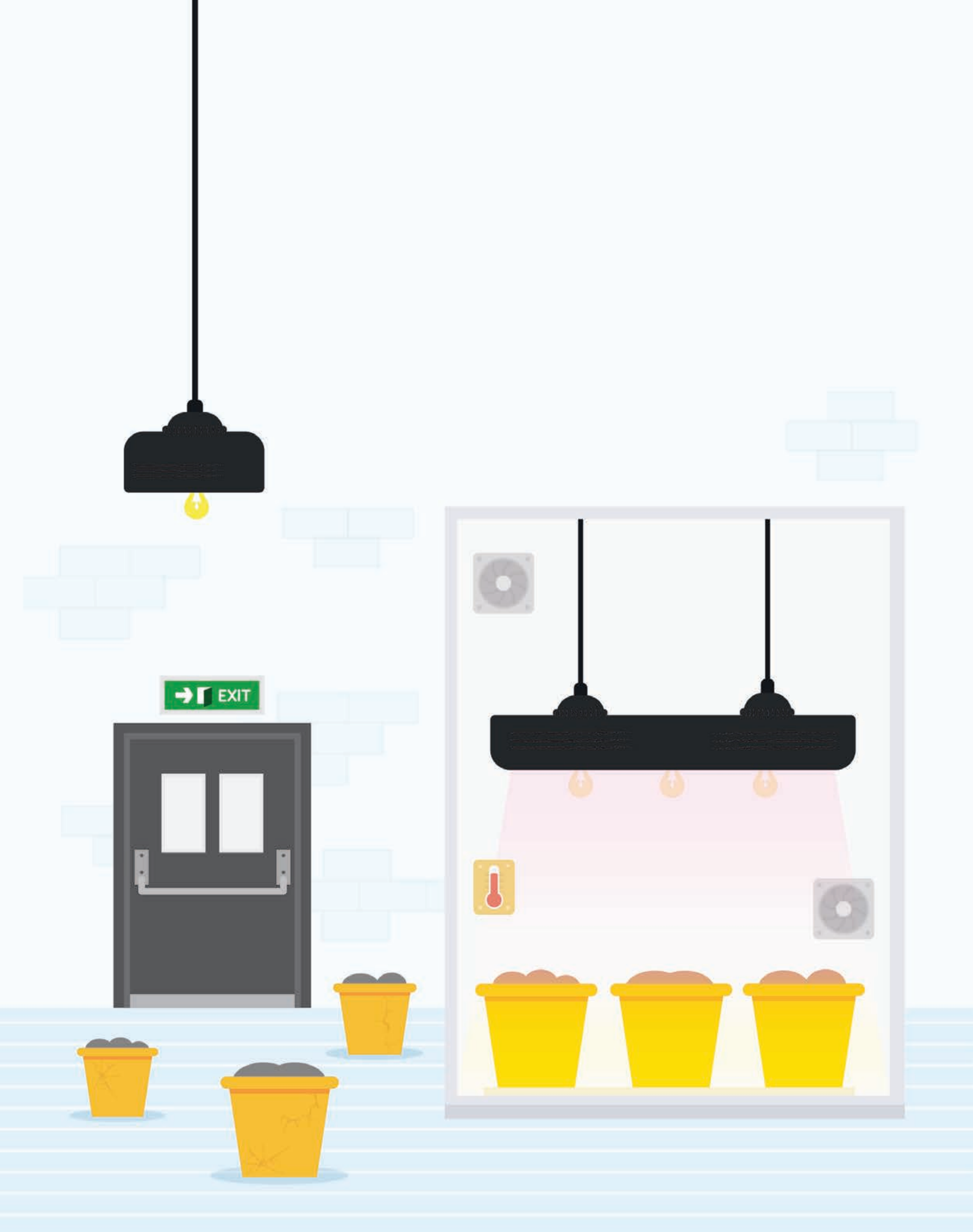

EXIT

THE *Cannabinoid* FACTORIES OF THE *Future*

Cannabis plants produce hundreds of interesting compounds, but could microorganisms provide a more efficient means for their mass production?

Synthia (formally known as *Mycoplasma laboratorium*) was the prodigal child of synthetic biology – a living cell comprising the outer membrane of a hollowed-out *Mycoplasma capricolum* and a *Mycoplasma mycoides* genome synthesized completely from digitized sequence information (1). This science may sound more aligned with the plot of a Philip K. Dick novel than reality, but synthetic biology was pushing these boundaries as early as 2010.

Since then, researchers have extended the frontiers of this novel field. *E.coli* cells have been engineered with the ability to produce synthetic proteins from a genetic code containing manmade nucleotide bases – essentially re-sketching the blueprints of life – and we have also equipped living cells with computational capabilities, such as logic, memory and problem-solving. Though impressive, these breakthroughs tell us very little about what synthetic biology is – or what it has to offer in practical terms.

Simply put, synthetic biology is an interdisciplinary field that applies engineering principles to the construction of biological systems that fulfil prespecified functions (for example, the synthesis of fuels or vaccines) based on carefully designed genetic circuits. The gene-editing approaches central to this field are adaptable, and are being harnessed to engineer microorganisms able to rival classical ways of providing commodities in many spheres.

In the medical cannabis industry, for example, microbes have been engineered with the capacity to produce a number of cannabis compounds – largely cannabinoids like cannabidiol (CBD) and cannabigerol (CBG), but also a number of further substances. Yet, these compounds represent only a fraction of the potentially therapeutic compounds found in the plant. In this sense (and others yet to be discussed), we have merely scratched the surface of synthetic biology's potential in this space.

The medical cannabis community has taken small steps towards a biosynthetic future – but what giant leap is needed before we usher in the age of cannabis' synthetic overlords? And why would we bother in the first place?

BIOSYNTHETIC SUPERIORITY?

Thinking on the latter question of why, Anna Shlimak, Head of Investor Relations and Communications for Cronos Group, provided a clear rationale. "The potential uses of cannabinoids are vast, but the key to successfully bringing cannabinoid-based products to market lies in creating reliable, consistent, and scalable production capability across the full spectrum of cannabinoids."

There are three basic ways to go about producing any compound at scale: synthetic chemistry, classical agriculture, or cellular agriculture (an umbrella term under which synthetic biology sits). When it comes to cannabis compounds, classical agriculture is clearly the historical method of choice, but the approach suffers from clear challenges. In fact, agricultural cultivation is burdened by a number of shortcomings.

"The agricultural approach is not economically effective or environmentally sustainable," says Jason Poulos, CEO of Librede Inc. "Hundred-thousand-foot greenhouses are needed to meet the demand for cannabinoids, which require not only colossal amounts of electricity to power, but also huge amounts of fertilizers and pesticides to produce a quality product." The negative effects of these compounds are widely documented, and span from depleting key insect populations to damaging human health and the environment.

"Producing these compounds in yeast, or another applicable microorganism, has the potential to reduce costs, stabilize the

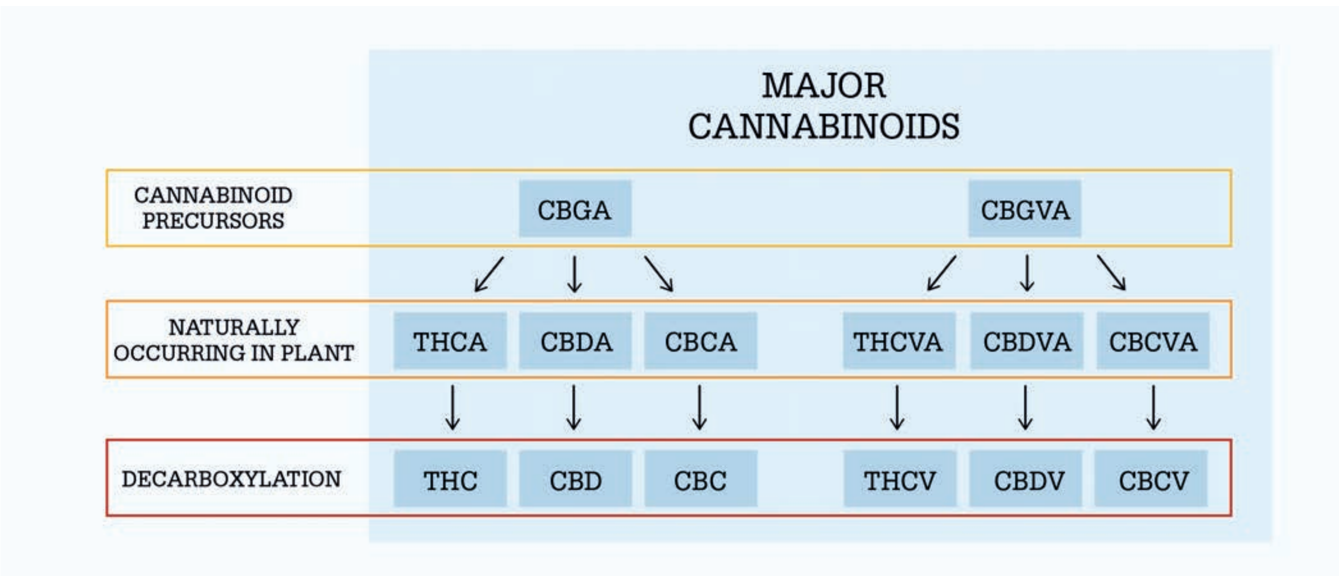
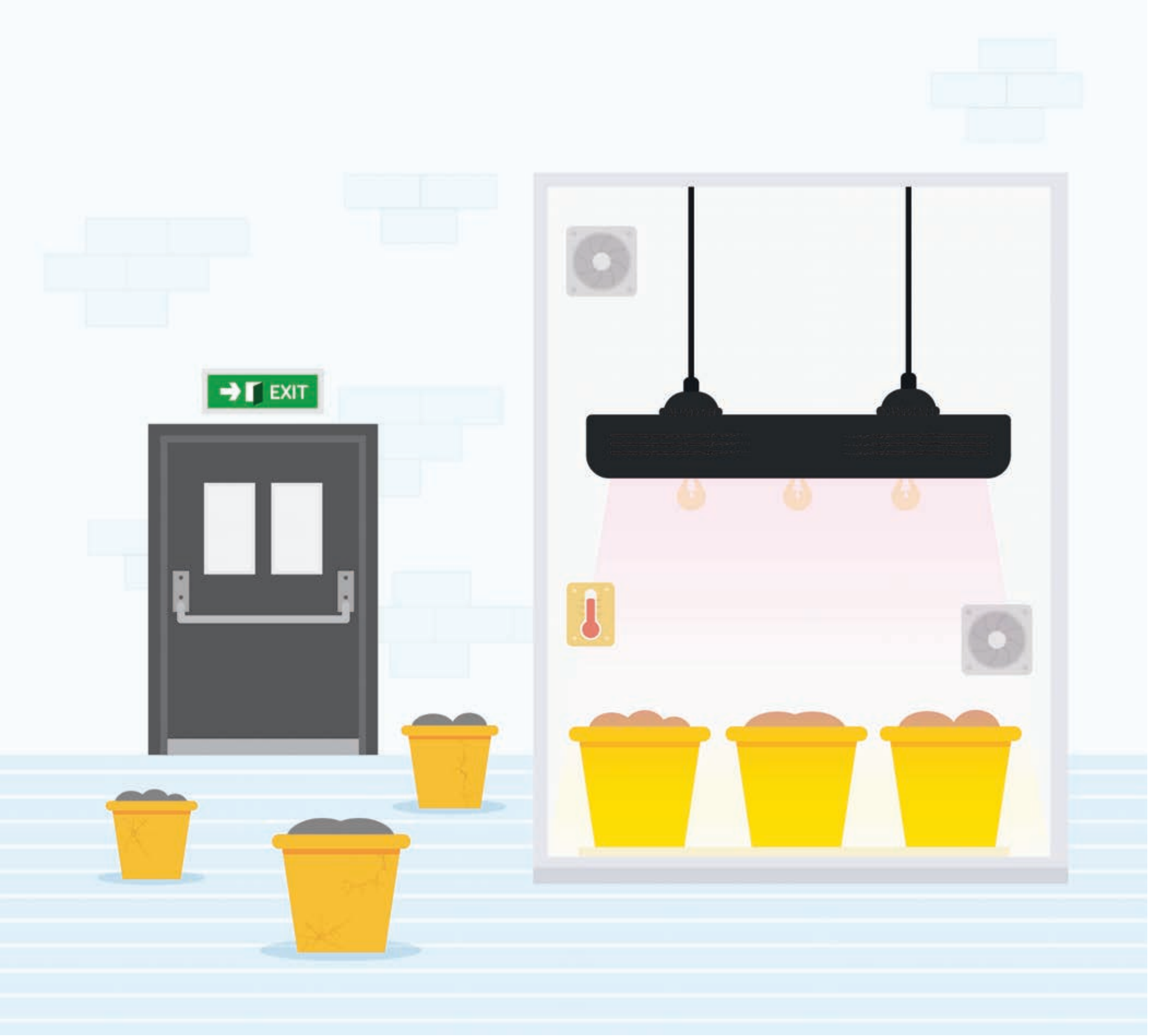


Figure 1. Cannabinoid metabolism in cannabis plants from CBGA and CBGVA "stem cell cannabinoids." Reproduced with permission from Cronos Group.



"The agricultural approach is not economically effective or environmentally sustainable."

supply chain against issues such as weather variability, and increase accessibility," says Poulos. "In the case of molecules like CBD, an anti-epileptic medicine, accessibility should be our primary concern."

Crop growth is affected by many factors, and even slight perturbations can lead to changes in the yield of a target compound by 10 percent or more. In the interest of stabilizing supply chains and increasing accessibility, as Poulos suggests, we must be able to anticipate the amount of a compound obtainable from a given production process.

Accessibility is a key theme in biosynthetic discussions – but expanding the portfolio of medical compounds available through cannabis represents another important goal. Biosynthesis allows us to capitalize on promiscuous metabolic pathways (those able to process one of a number of substrates into different products) to produce unnatural analogues of target compounds; the cannabinoid pathway can produce many such analogues when fed variants of hexanoic acid.

Talking of these possibilities, Jay Keasling of the University of California, Berkeley, suggests that synthetic biology carries the major advantages of both synthetic chemical and biochemical manufacturing approaches in a single package. "The great thing about synthetic biology is that it provides a relatively simple route for producing natural molecules with stereochemical centers, and also facilitates the synthesis of molecular variants," he says. "This covers some of the weak points of synthetic chemistry and biochemical methods, respectively."

THE BIOSYNTHESIZERS

Jay Keasling

A household name in the field of synthetic biology, Jay Keasling has spent 27 years at University of California, Berkeley, engineering microbes. The success stories in his portfolio? Biosynthesizing taxol (an anti-cancer drug) and artemisinin (an anti-malarial drug precursor), and an extensive number of biofuels. Keasling has also been involved in numerous business ventures, co-founding both Amyris and Lygos. The recipient of numerous awards in both bioengineering and innovation, Keasling says he is looking forward to focusing on increasingly elusive molecules as he embarks on future ventures including the biosynthesis of key cannabis compounds.

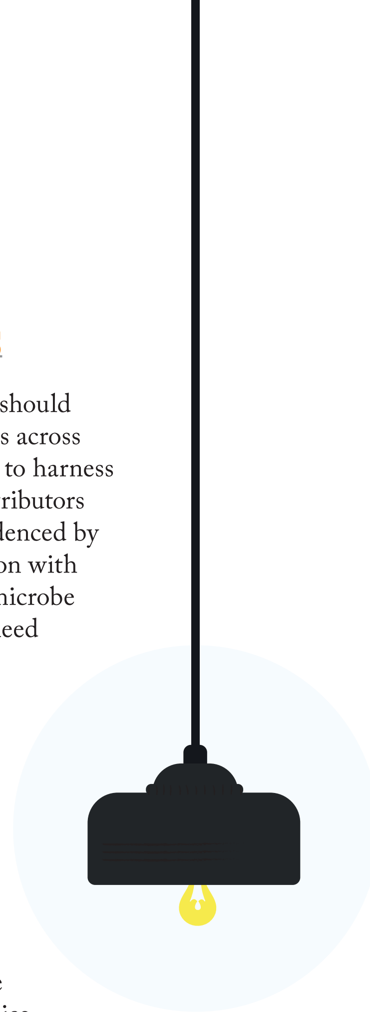
Anna Shlimak

Head of Investor Relations and Communications at Cronos Group – a global cannabinoid company committed to building disruptive intellectual property by advancing cannabis research and product development – Anna Shlimak plays a crucial role in communicating the company's cannabinoid biosynthesis programs. Working with Ginkgo Bioworks, they are making waves in the field of cannabinoid biosynthesis by using the expertise of both organizations to tackle key issues, such as scalability, access to rare cannabinoids and economic sustainability.

Jason Poulos

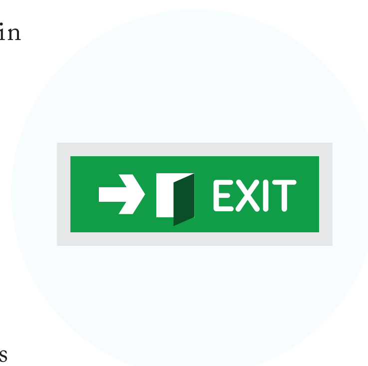
Librede is a company with a clear goal: to harness the therapeutic potential of nature. Cannabinoids represent valuable potential in this endeavor, providing a window of opportunity that CEO Jason Poulos was not prepared to miss. After obtaining his PhD in bioengineering from the University of California, Poulos wasted no time in immersing himself in the upper echelons of biotech business, soon developing the world's first yeast-based cannabinoid production platform alongside Anthony Farina. Today, his focus is on establishing a wide network of collaborators with whom to develop new methods for synthesizing molecules.

RISE OF THE BIOSYNTHESIZERS



With such advantages in mind, it should come as no surprise that big names across industry and academia are hoping to harness this untapped potential – our contributors included. For Shlimak, this is evidenced by Cronos Group's recent collaboration with Ginkgo Bioworks (a customized microbe design company); for Poulos, we need only look at Librede's history of biosynthetic cannabinoid patents; and for Keasling, recent success in synthesizing cannabinoids and their unnatural analogues in yeast speaks for itself (2).

Accordingly, Poulos highlights extreme levels of competition. "Every day there seems to be a new company wanting to enter this space. We're even seeing publicly traded companies take interest, but these groups quickly realize that saying is easier than doing," he says. Librede is developing a hefty patent portfolio to beat off competitors; the most recent of these covered an approach for producing THC acid (THCA) in yeast, with existing patents in place for methods concerned with the biosynthesis of CBD and CBG. The existence of so much competition so soon after changes to legislation points to rapid and continued progress for cannabinoid biosynthesis in the industrial sphere.



But industry is not alone in taking such strides. Keasling and his team published their method for the complete biosynthesis of major cannabinoids (cannabigerolic acid [CBGA], THCA, cannabidiolic acid, Δ9-tetrahydrocannabivarinic acid and cannabidivarinic acid [CBDVA]) in yeast using a galactose substrate in February. Keasling says we've come a long way. "Such research was stuck in the Dark Ages 20 years ago! No internet and no sequenced genomes – you did well just to get one gene into an organism. Today, we are working on pathways containing 35 plant genes."

> "Such research was stuck in the Dark Ages 20 years ago! No internet and no sequenced genomes – you did well just to get one gene into an organism. Today, we are working on pathways containing 35 plant genes."

Feats elsewhere in academia also speak to a rapidly advancing subfield. A team from TU Dortmund University (Germany) have engineered another species of yeast with the ability to conduct whole-cell CBGA to THCA bioconversion (3), and, in August 2019, Kevin Rea and colleagues from the University of Guelph (Canada) reported the successful biosynthesis of cannabis anti-inflammatories cannflavin A and B, also in yeast (4).

Keasling attributes these advances to a number of breakthroughs in molecular science, including DNA synthesis. Labs today are able to bypass cloning genes out of the cannabis plant completely, and can also alter the codons within to modify genetic expression as required. What's more, automation and robotics streamline the practical work itself, and dramatic advances in analytical technologies like mass spectrometry mean that we can analyze the biosynthesized products with high throughput.

With the aim of capitalizing on such advances to biosynthesize eight target cannabinoids, Cronos Group have recently purchased a state-of-the-art facility, which will operate as "Cronos Fermentation." Shlimak was happy to share a few details. "The facility includes fully equipped laboratories covering microbiology, organic and analytical chemistry, quality control and method development, as well as two large microbial fermentation areas with a combined production capacity of 102,000 liters. Plus, three downstream processing plants, and bulk product and packaging capabilities," she says.

Such facilities should provide the fermentation and manufacturing capabilities needed for Cronos Group to take full advantage of the work currently underway with Ginkgo. "Simply put, the process will be similar to that of yeast-based beer fermentation," says Shlimak, but current gaps in our knowledge of cannabinoid biosynthesis and the relevant technologies mean we have a way to go before upscaling to volumes as striking as 102,000 liters.

BLOCKERS TO BIOSYNTHETIC SUCCESS

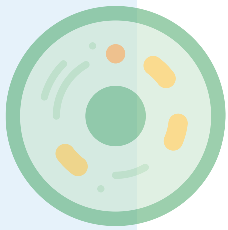
To upscale production, those in pursuit of cannabis compound biosynthesis must focus their expertise and ingenuity on three

NEXT-GENERATION YEAST

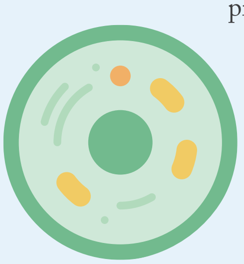
From bread to beer and fuel, yeast plays a key role in manufacture. Now cannabinoids join the list.

The cannabinoid biosynthesis race is as close as ever, and notable breakthroughs are popping up across all corners of the research world. Here, we take a deeper look at the method for biosynthesizing cannabinoids and their unnatural analogues in yeast from Jay Keasling's lab.

The team, led by lead author Xiaozhou Lou, engineered the native mevalonate pathway of *Saccharoymyces cerevisiae* (brewer's yeast) and introduced a hexanoyl-CoA pathway constructed from the genetic components of multiple bacterial species to achieve cannabinoid synthesis using a simple galactose substrate (1). Of course, genetic elements of the cannabis plant itself were also used – in particular, those encoding enzymes involved in olivetolic acid synthesis.



The researchers were also able to capitalize on promiscuous pathways to produce potentially useful compounds not found in the native plant. The team were also able to identify a previously uncharacterized enzyme and new genes encoding cannabinoid synthases during their efforts. "All the necessary pathway enzymes had supposedly been discovered, but we soon found that a previously documented prenyltransferase wouldn't work as needed partway through the study. This is a really critical step, and so we had to go back into the cannabis plant to find another," says Keasling.



The work represents an approach that could overcome many of the difficulties associated with pure chemical synthesis, including the high structural complexity of the molecules in question. The overall aim is to enhance healthcare for those who need it… But time will tell as to how these methods measure up against existing approaches.

Reference

1. *X Luo et al., "Complete biosynthesis of cannabinoids and their unnatural analogues in yeast", Nature, 576, 123-126 (2019). DOI: 10.1038/s41586-019-0978-9*

significant objectives: improving technology, reducing costs, and championing success stories.

Keasling believes that translating a biosynthetic cannabinoid product from laboratories to the market will be a crucial first step. "Getting a product into the market and placing it into the hands of consumers is essential because it will act as proof of concept to the public and evidence that this area of research was worth the investment," he says. Such success stories would likely then spur on further research; as Shlimak says, "Providing consistent and reliable products will facilitate additional innovation in this space by bringing new formats and technologies to the market."

A change in public perception would also help. The term genetically modified organism seems to incite confusion and fear even today, and an improved public understanding would ease the movement of biosynthesized cannabinoids into the market.

In terms of improving technology, Poulos believes the field would benefit most from advances that focus on screening for the cannabinoids being biosynthesized, rather than on the genetic means for their production: "We can easily incorporate enzymes from countless organisms into a microbe of interest by simply printing the DNA encoding them. The problem is: how do we quickly test that the resulting cannabinoids are really there? It's not as simple as observing a change in color."

Poulos argues that such screening tools could be useful not just for cannabinoid biosynthesis, but also in biosynthetic efforts to produce classical pharmaceutical products, biofuels, and so on. "If I could snap my fingers tomorrow and have any breakthrough in this field in front of me, improved screening would be it," he says.

In addition, advances in the development of stable cell lines suitable for use on an industrial scale and downstream processing for cannabis compound extraction and purification are also needed. Low-cost materials will be central to these endeavors, which underscores perhaps the most important requirement for the field moving forward – reduced costs.

Though the chemical synthesis of cannabis compounds is incredibly expensive, costing around $40,000–70,000 per kilogram, Keasling suggests that costs as low as $100 per kilogram could be accomplished through microbial synthesis. With a wholesale price for CBD of around $5,000 per kilogram, reducing the price of biosynthesis represents a route to maximize yield and profit for the companies working in this space. Perhaps the most lucrative line of attack, however, will be the biosynthesis of lesser-known cannabis compounds with medical applications, which currently hold wholesale values of up to around $60,000 per kilogram.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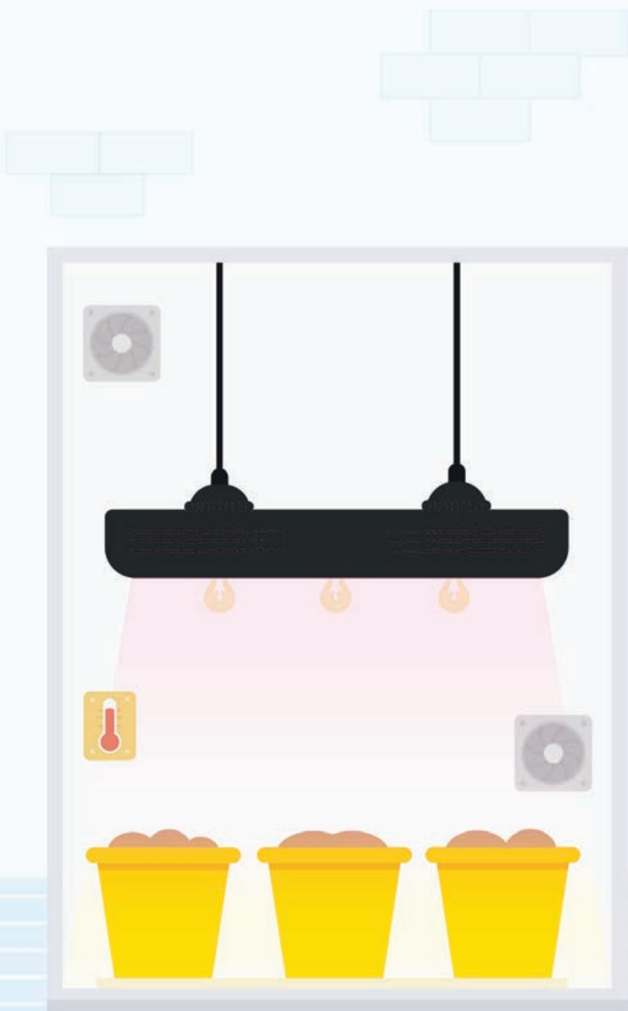
"We can easily incorporate enzymes from countless organisms into a microbe of interest by simply printing the DNA encoding them."



BIOSYNTHETIC BLOCKBUSTERS OF TOMORROW

Cannabis compounds other than those typically mentioned in medical discussions are an important target for biosynthesis. Keasling refers to these as potential "blockbuster" compounds, and is excited to explore the utility of yeast to synthesize them. "I'm really interested in rare cannabinoids, but we don't know much about them because of the small quantities they're produced in. Yeast provides a great platform to further study these compounds, and I'm really excited to see these experiments unfold."

These compounds also represent an important target for Cronos Group. "Our platform with Ginkgo will hopefully grant us access to cannabinoids present at low quantities in the plant, meaning that they are economically impractical, difficult or impossible to extract from agricultural sources. These could be medically important, and potentially very valuable," says Shlimak.

For Poulos, future ventures will focus on so-called "stem cell cannabinoids" CBGA and cannabigerovarinic acid – major cannabinoid precursors (see Figure 1 for an overview of their metabolism). Librede is already producing these compounds in quantities of hundreds of grams and is focusing on process improvements to lower costs. Next on their hit list: further compounds such as THC, CBD, cannabichromene, and tetrahydrocannabivarin.

Despite the potential and value of lesser-known and little-understood cannabinoids, the current drive of the field is clear. "From a pharmaceutical standpoint, the primary molecule of interest is CBD right now," says Poulos.

Beyond pharma, we should also expect to see applications for biosynthesized cannabinoids in nutraceuticals, beer, cosmetics, and any other markets that deem them useful. The demand for this research is clear – now it's up to researchers to continue pushing the boundaries.

Will we see cannabis greenhouses replaced completely by fermentation tanks? Maybe not anytime soon. But the case for cannabinoid biosynthesis – both in mass production and drug discovery – is certainly compelling.

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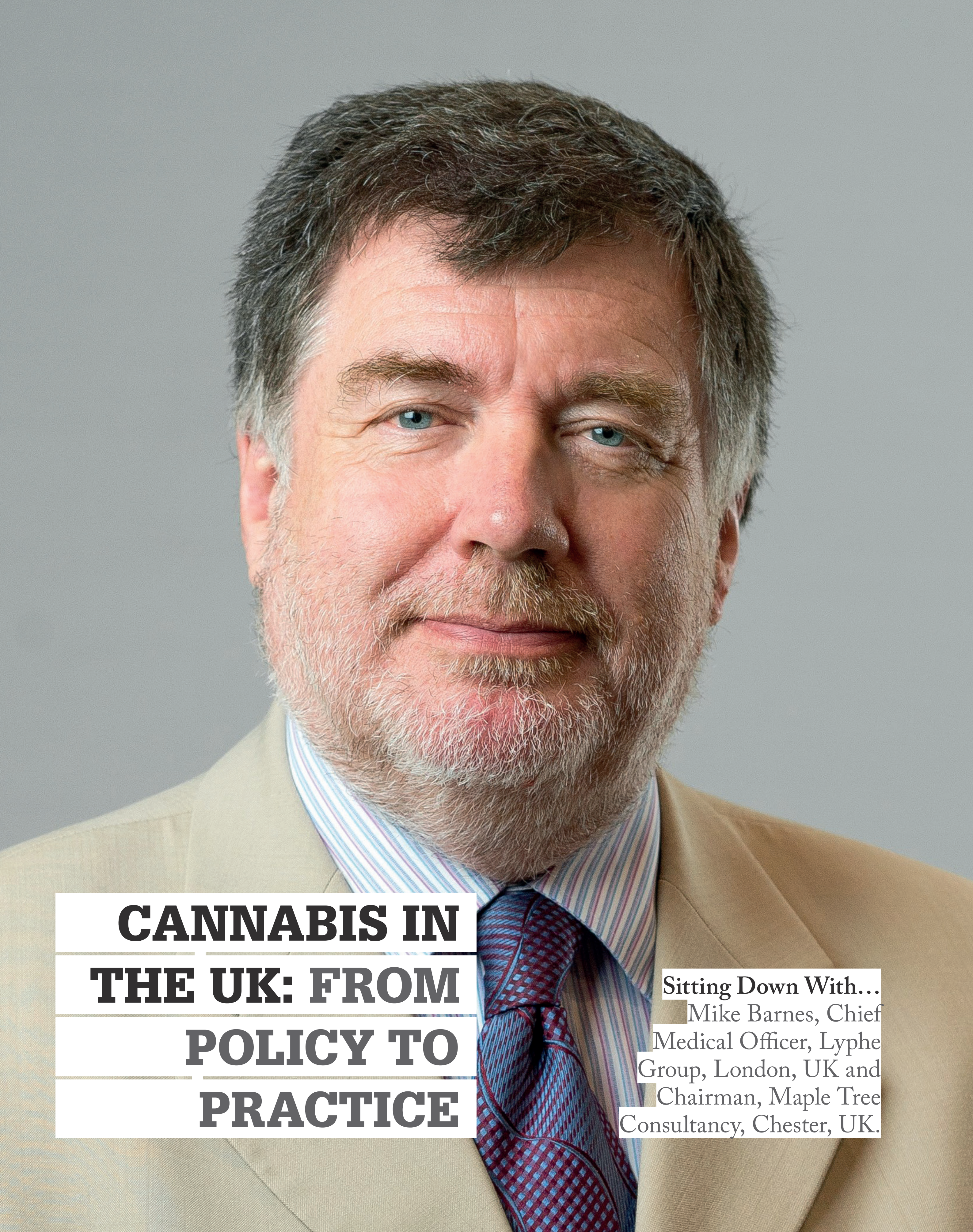

CANNABIS IN THE UK: FROM POLICY TO PRACTICE

Sitting Down With…
Mike Barnes, Chief Medical Officer, Lyphe Group, London, UK and Chairman, Maple Tree Consultancy, Chester, UK.

How did you get involved in the medical cannabis field?

I began my career as a neurologist, with particular interest in how the brain recovers from neurological injury and long-term conditions like multiple sclerosis (MS). Two decades ago discussions were well underway, exploring whether cannabis could relieve MS-associated pain. I was intrigued; if thousands of patients were willing to commit a crime to obtain and use these cannabinoid-inspired products, then perhaps it was worth investigating!

Throughout the early 2000s I was involved in the development and subsequent licensing of Sativex, which remains the only cannabis-based product for MS spasticity. Unfortunately, the National Institute for Health and Care Excellence (NICE) later declared that Sativex was not cost-effective, and therefore couldn't be prescribed. I went quiet until 2016, when the all-party parliamentary group on drug policy asked me to conduct a review of the efficacy of medicinal cannabis. Alongside my roles in a number of advocacy campaigns (fighting to gain licenses for particular patients), I found myself thrust into the media spotlight.

Why is medical cannabis failing to gain traction in the UK?

Our doctors aren't trained in medicinal cannabis; they've not studied the endocannabinoid system, nor do they understand medical implication, form, or dosage. In tandem, The Royal College of Physicians and the British Paediatric Neurology Association both provide remarkably negative coverage. They claim there isn't sufficient evidence to prescribe medicinal cannabis for pain treatment – which flies in the face of significant evidence gathered in the 50 or so countries where medicinal cannabis has been legalized. Education, coupled with changes in policy are required before we see any meaningful change.

What part does government play?

Bureaucracy is certainly a problem. Prescriptions require the go-ahead from a hospital doctor – you can't obtain medicinal cannabis from your family GP. The hospital health trust must gain permission from further up the hierarchy; funding is required, and there are several examples of cases creeping past these bottlenecks only to be denied by the clinical commissioning group. The government is hiding behind the legislation. It's all well and good to say "we've changed the law, it's up to doctors to prescribe," but there is a lot they could do to help. Improving the supply chain is an important goal, but what's really needed are good-quality health economics studies. Once we can demonstrate the cost-effectiveness of medicinal cannabis, progress will surely follow.

Government policy alone won't be sufficient to change perceptions, education is equally important. Many doctors are refusing to engage in conversation – convinced medicinal cannabis is a fad. Others are more tuned in, but have been prevented from prescribing it by hospital hierarchies. We established the Cannabis Clinic Academy to provide a basic level of training for all GPs. It combines three days of theoretical background knowledge – history, endocannabinoids/terpenes and medical products – with the practicalities of prescribing. We also provide hands-on mentoring, ensuring support and reassurance as doctors begin to put in practice what they have learned.

Your book, A Beginner's Guide to Medicinal Cannabis, was released in April. Tell us about that.

I'd been thinking about the need for a simple, easy-to-understand guide to medicinal cannabis for years. Like most things in life, however, it remained a pipedream! I was approached by the publishers Berrybooks, who asked me if I thought there was a market interest. I said yes; the result is a book written in the space of about a month, containing about two hours of reading. It's really a starting point for those with no knowledge of the industry. The response has been overwhelmingly positive. The next project will be a more advanced tome, but I don't yet have the energy – or time – to get to work on that one!

"Many doctors are refusing to engage in conversation – convinced medicinal cannabis is a fad."

Dare we dream of a more optimistic future?

I certainly hope so! Canada, Australia, Germany, and many others are far ahead of us – both in terms of legislation and public debate. I hope we can follow a similar path, with British doctors beginning to prescribe medicinal cannabis in larger numbers. Perhaps they'll think: "Hang on a minute, why are we so reluctant to prescribe? In other countries these products are working well." Things are changing, with an uptick in interest for our Cannabis Clinics and broader public awareness. Cracks are appearing in the dam; I'm hopeful it will burst in 2020, ushering in a significant upturn in prescription volume.

As for my own plans? Probably retirement! I'm nearly 67, so at some point I'd like to slow down. In the short term, however, cannabis projects are all-encompassing. Over the next few years I'd like to see more Cannabis Clinics established; at the same time, I'd like our educational initiatives to take on a more global perspective. I'm determined to keep working until medicinal cannabis is a well-established prescription on the UK National Health Service.