



# Investigating a not-so-natural high

Researchers identify synthetic cannabinoids in herbal incense.

Would you do something wrong if you knew you weren't going to be caught? This prospect tempted thousands of people as word spread about a "legal high"—herbal incense that could be smoked like marijuana. Researchers in several countries failed to find evidence of any common psychotropic compounds when they tested these herbal mixtures. And the urine of people who appeared to have overdosed on the substance did not contain known drug metabolites. But with a lot of analytical chemistry, some Internet research, and a little serendipity, researchers in Germany eventually identified synthetic cannabinoids in several different types of herbal incense; they recently reported their results in the *Journal of Mass Spectrometry* (2009, DOI 10.1002/jms.1558).

## A public service announcement backfires?

Reporters for German television probably thought that they were doing society a favor when they broadcast a news story about herbal incense that produced a marijuana-like high but couldn't be detected by common drug screens. But after the August 2008 broadcast, the popularity of the incense soared in Germany. Young people began to show up at emergency rooms across the country with psychosis-like panic attacks and heart and circulatory problems; these patients admitted to smoking or ingesting products marketed as "herbal incense". Despite all signs pointing to marijuana overdose, no delta-9-tetrahydrocannabinol (THC) or any of its metabolites were detected in clinical samples from the patients.

## The guinea pigs

Volker Auwärter's laboratory at the Institute of Forensic Medicine at the

University Medical Center Freiburg (Germany), together with the Bundeskriminalamt (BKA), Germany's federal

from all the symptoms—starting from red conjunctivae, dry mouth, and very high pulse rate," says Auwärter. But again, the scientists did not detect THC or other common psychotropic substances in the clinical samples. The researchers did, however, see some unidentified peaks in the GC/MS analysis of blood; these peaks were traced back to unidentified substances also detected in extracts of the herbal mixtures.



JULIE FARRAR/PHOTODISC

criminal police office, performed more extensive testing for psychotropic compounds in the clinical samples from the overdose patients but found nothing. Baffled, Auwärter and colleagues decided to do a controlled self-experiment with one type of herbal incense so they would have clinical samples after a known consumption of the substance. "First, we wanted to know is there a real effect, and if there is a real effect, there has to be a substance that is responsible for it," says Auwärter. The group wanted to collect enough clinical samples over an extended period to be able to perform preliminary kinetic analyses on any metabolites, which are often the only compounds detectable in urine.

Much to the researchers' surprise, a low dose (0.3 g of the mixture smoked by two of the researchers) produced a psychotropic effect that lasted for 5–6 hours. "It was really cannabis-like

## Synthetic cannabinoids

Next, the investigators turned to the Internet, where they found rumors that the herbal mixtures had been spiked with synthetic compounds that could not be detected by drug screens. So the group shifted focus and began to look for synthetic cannabinoid agonists. The researchers tested seven different products and analyzed extracts from the herbal mixtures via GC/electron impact MS, LC/MS<sup>n</sup>, UV spectroscopy, and TLC, but they still couldn't determine the identity of three peaks. Auwärter points out that the laboratories could not identify the unknown compounds quickly because the compounds weren't in any of their mass spectra libraries.

According to Auwärter, a critical step in the group's analyses was the isolation of milligram quantities of the unknowns via silica gel chromatography so that they could perform structural analysis by NMR. "We had some information from each method, and that had to be puzzled together," he says. For example, TLC of extracts from three varieties of one brand of incense showed that the amount of two unknown compounds increased with the price of the product; this was a pretty good indication that the com-

pounds were intentionally added to the herbal mixtures. Eventually, the group pieced together a structure for one of those unknowns, but the structure was not related to THC.

### Serendipity knocks

In mid-December 2008, the collaborators were still trying to deduce the structures of the unknown compounds when they got lucky. A commercial laboratory in Frankfurt announced that it had identified JWH-018, one of several cannabinoid agonists synthesized at Clemson University, in samples of herbal incense. The city of Frankfurt had asked THC Pharm, a company producing pharmaceuticals based on natural cannabinoids, to analyze the herbal incense. The firm's scientists were able to match the GC/MS spectrum of one peak to spectra they'd previously collected on JWH-018. Auwärter says that the Frankfurt laboratory did see another unidentified peak in the GC/MS spectrum. "They didn't know what it was, but they just suspected it may be some kind of aroma" component, he says.

Armed with this new knowledge, Auwärter and colleagues combed through the literature on other synthetic cannabinoids, such as the CP and HU series of compounds synthesized at Pfizer and Hebrew University (Israel), respectively. The group quickly matched its data with published data on cannabinoid agonists. By comparing the NMR spectra, the scientists realized that the structure they had previously pieced together was a homologue of CP 47,497—a compound known to bind to the brain cannabinoid receptor. In the seven products tested, the researchers identified CP 47,497; the C8 homologue of CP 47,497 and its trans diastereomer; JWH-018; and oleamide. The CP 47,497 homologue and its diastereomer were the major components for five out of the seven products tested; for the other two products, JWH-018 was the major component.

The nature of the herbal incense products has changed over time. Originally, Auwärter thought that the first



One of the herbal mixtures tested, Spice Gold.

products sold did not contain cannabinoids. But after laboratories knew what to look for and reanalyzed the earlier batches of incense, they detected the spiked cannabinoids. Auwärter says, "It seems like the labs doing the analyses at that time were just not suspicious enough." And batch-to-batch reproducibility was an issue as well. In one brand of herbal incense, sometimes JWH-018 was detected and sometimes it wasn't. After publishing their paper, the researchers found yet another synthetic cannabinoid in a batch of incense.

But the spiked compounds are not the only thing changing. "In the beginning, the Swiss labs were able to find at least some of the herbs which are declared on the back side of the package," says Auwärter. He says that by the time the herbal mixtures laced with cannabinoids became popular in Germany, the mixtures did not even contain the herbs listed in the ingredients. "[The manufacturers] just took any kind of plant material that is cheap," says Auwärter.

### Going global

Starting in December 2008, countries including Austria and Switzerland banned the herbal incense. Auwärter's group and the BKA issued a press release about their findings in mid-Janu-

ary 2009. Within days, Germany banned CP 47,497 and its pharmacologically active homologues and JWH-018. France outlawed all of those compounds plus another synthetic cannabinoid, HU-210, in February 2009.

But spiked herbal incense is not just a European phenomenon. The U.S. Customs and Border Protection (CBP) announced in mid-January 2009 that it had seized herbal incense shown to contain HU-210. When CBP officers at the Ohio facility of an international express courier saw the dried plant material inside a shipment, they performed a field test for the presence of THC, but it was negative. Further analysis at the CBP laboratory in Chicago confirmed the presence of HU-210.

Over a three-month period, CBP seized >100 lb of HU-210-laced herbal mixtures in five separate shipments. (Interestingly, a narcotics detector dog led authorities to the substance in at least one of the cases.)

Brett Sturgeon, a CBP public liaison officer, says that the agency has made seizures of this substance in other U.S. ports as well. And a couple of weeks after the CBP announcement, scientists at Japan's National Institute of Health Sciences published a paper in the *Chemical and Pharmaceutical Bulletin* (2009, 57, 439–441) identifying the same C8 homologue of CP 47,497 in herbal incense; they did not report the presence of JWH-018 or the diastereomer that Auwärter's group found, however.

### How do they do it?

Auwärter hypothesizes that the producers buy cannabinoids from laboratories in China or other countries that offer cheap organic syntheses, dissolve the compounds in a solvent, spray the solution on the plant material, and evaporate the solvent before packaging the herbal mixtures. At a price of 20–30 euros (~\$25–40) for a 3-g packet, the incense is significantly more expensive

COURTESY OF VOLKER AUWÄRTER

than marijuana. Nevertheless, its popularity has spread—probably in no small part because word travels quickly on the Internet.

How do the vendors sell so much of their product without getting caught? “I guess it’s the same like with all these pharmaceutical products like Viagra that are sold over the Internet,” Auwärter says. “They have a whole system of distribution. Commonly, they have a base overseas ... and from there, they send it from one country to the other. It’s very difficult

for national authorities to trace back the whole thing.”

So the group next plans to do profiling experiments to help pinpoint the source(s) of the synthetic compounds. “Now, almost daily, we get new products which are also declared as herbal incense, with different names, different packaging.... Always, you have some plant material and sometimes you have cannabinoids that are already known,” says Auwärter. “And of course, there are some products which do not contain any pharmacologically active

compounds, which are just fakes.” The researchers will also work on finding metabolites in urine that could be used to detect consumption, even days after the last intake. But with such a variety of synthetic cannabinoids, that will be a challenge. “Now that JWH-018 is controlled, then next on the market may be ‘butyl-’ or ‘hexyl-’ instead of ‘pentyl-’ and so on,” says Auwärter. “[Tracing these compounds is] now a rat race.”

—Christine Piggee