**Vaping Cannabinoid Acetates Leads to Ketene Formation**

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**ABSTRACT**

$\Delta^8$-THC acetate is a relatively new psychoactive cannabis product that is available online and in vape shops across the US since it is currently unregulated. Because it contains a similar substructure to vitamin E acetate, which has been shown to form the poison gas ketene during vaping, we investigated potential ketene formation from $\Delta^8$-THC acetate, as well as other cannabinoids acetates, CBN acetate and CBD acetate, under vaping conditions. Ketene was consistently observed in vaped condensates from all three acetates as well as from a commercial delta-8 THC acetate product purchased online.

**INTRODUCTION**

THC acetates are semi-synthetic psychoactive cannabinoids obtained via acetylation of the cannabinoid phenol moiety.[1] The acetylation reaction is analogous to that used for the transformation of morphine to heroin,[2] to afford increased lipophilicity and blood brain barrier permeability.[3] $\Delta^9$-THC acetate ($\Delta^9$-THC-OAc, or THCO in some sources, Figure 1) had been investigated as an incapacitating agent by the US military as part of the Edgewood Arsenal experiments between 1949 and 1975 and was found to produce a two-fold greater degree of ataxia in dogs compared to $\Delta^9$-THC.[4] Recent anecdotal reports from users state that $\Delta^9$-THC-OAc has significantly more psychoactive potency (*ca*. 300 % more) than THC. The legal status of $\Delta^9$-THC-OAc is currently unclear. $\Delta^8$-THC, an unregulated isomer of $\Delta^9$-THC, along with its acetate derivative ($\Delta^8$-THC-OAc, Figure 1), have become increasingly available, particularly in states where $\Delta^9$-THC is illegal.[5] However, there is a lack of published peer-reviewed research concerning the cannabinoid acetates. The health risks of these compounds are currently unknown, despite their potency and ready availability.

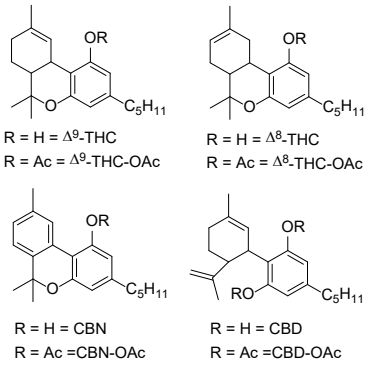
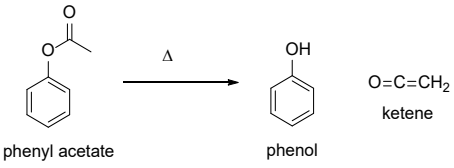



**Figure 1.** Selected cannabinoids (R = H) and their *O*-acetyl (acetate) derivatives (R = Ac).

Each of the cannabinoid acetates possesses a phenyl acetate substructure. Phenyl acetate has been known since 1938 to convert to ketene and phenol under thermolytic conditions (Scheme 1).[6] Ketene is a highly reactive poisonous gas. NIOSH has established 5 ppm as the immediately dangerous to life or

health (IDLH) value for ketene.[7] In 2019, Wu and O'Shea reported the formation of ketene from vitamin E acetate when vaping with a commercial electronic cigarette,[8] leading to ketene's consideration as a possible causative agent of e-cigarette or vaping use-associated lung injury (EVALI).[9] Since cannabinoid acetates and vitamin E acetate both contain phenyl acetate moieties, we hypothesized that vaping cannabinoid acetates could lead to ketene exposure.




Scheme 1. Phenyl acetate converts to phenol and ketene under thermolytic conditions.

**EXPERIMENTAL SECTION**

**Materials and methods.** Samples of CBN-OAc and CBD-$OAc_2$ were synthesized by Dr. Rob Jensen and supplied by FloraWorks™. The CBN-OAc (99%) CBD-$(OAc)_2$ (95%) were used as received. A commercial formulation containing $\Delta^8$-THC-OAc was purchased from Hydro-Hemp in a pre-packaged SICKO™ brand vape cartridge and used at 10 W. Analysis of the commercial sample was performed using a capillary GC column mounted in an Agilent (Santa Clara, CA) 7890A GC interfaced to an Agilent 5975C MS, and operated in electron impact ionization mode. The sample contained approximately equimolar amounts of $\Delta^8$-THC-OAc and $\Delta^8$-THC, in addition to terpenes. Nuclear Magnetic Resonance (NMR) spectra for all dabbed/vaped acetate samples were acquired on a Bruker Avance III 600 MHz NMR spectrometer at 128 or 256 scans, 16 s relaxation delay for qNMR samples, 30-degree flip angle acquisition. Methyl 3,5-dinitrobenzoate was used as the qNMR internal standard.

**Dabbing experiments.** An electronic nail ("e-nail") was used in the dabbing experiments (EKS REX-C100 controller). The e-nail consists of a metal wire that is wrapped numerous times around the quartz nail and connected to the power source. Temperatures for dabbing experiments were set at 500 °C initially to screen for the presence of reacted ketene as *N*-benzylacetamide. For quantitative studies the e-nail temperature was set to 378 °C, consistent with our prior dabbing studies based on a survey of social media showing a range of preferred e-nail user temperatures of 340 – 482 °C.[10] Tygon tubing (1/4") connected the dab rig to an impinger that was connected to an SCSM-STEP Smoking Machine (CH Technologies). The impinger was filled with $CDCl_3$ (2 mL) for NMR analysis. Additionally, 150 μl of benzylamine (ketene trapping agent) and 100 μl of the internal standard for NMR (methyl 3,5-dinitrobenzoate) were included in the impinger. Trials were run using a modified version of the CORESTA protocol at a puff depth set at 1.32 and a draw time of 4.8 s, with 30 s between draws.

**Vaping experiments**. A LUXE S mod kit was used for the vaping experiments. All trials were run at 10 W which was within the manufacturers designated usage range. The pre-packaged SICKO™ brand vape cartridge containing $\Delta^8$-THC-OAc was connected to the battery and vaped into tygon tubing (1/4") connected to an impinger containing the same trapping solution as was used in the dabbing trials. An SCSM-STEP Smoking Machine (CH Technologies) was connected to the impinger and used as the vacuum source. Trials were run using a modified version of the CORESTA protocol using a puff depth set at 1.32 and a draw time of 3.0 s, with a 30 s interval between draws.

**RESULTS**

We investigated the formation of ketene from CBN-OAc, $\Delta^8$-THC-OAc and CBD-$(OAc)_2$ initially under "dabbing" conditions. Dabbing involves flash vaporization on a hot surface and exposes users to relatively large volumes of aerosol.[10] Unlike the use of vape cartridges, dabbing is less impacted by

sample viscosity, thereby enabling pure cannabinoids to be efficiently vaped without the need for confounding viscosity-modifying additives. [11, 12]

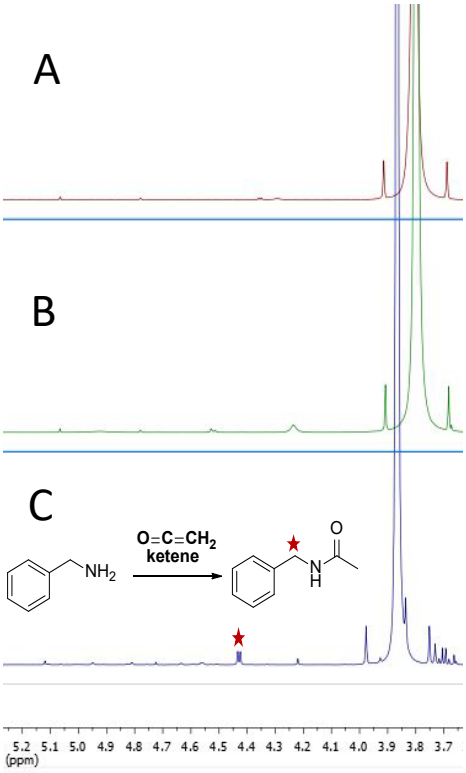



**Figure 2.** $^{1}$H NMR spectra of: **A)** unvaped CBN-OAc; **B)** vaped CBN and **C)** vaped CBN-OAc. All samples contained benzylamine. The star indicates the doublet (4.43 ppm) corresponding to the *N*-benzylamide methylene peak. *N*-benzylamide is formed via the reaction of ketene and benzylamine, and is only observed in the vaped CBN-OAc sample.

CBN-OAc was used for the initial studies as it produces fewer degradation products upon vaping compared to other cannabinoids,[12] and should therefore afford relatively cleaner NMR spectra. The quartz nail was set to a temperature of 378 $^{o}$C. The condensate from vaped CBN-OAc was collected in an impinger filled with $CDCl_3$ for seamless transfer to an NMR tube and quantitative NMR (qNMR) analysis. We had previously validated a qNMR technique for identifying and quantifying molecular components of

e-cigarette condensates.[13] Ketene was determined via trapping with benzylamine to afford *N*-benzylacetamide (Figure 2C).[8] As shown in Figure 2, *N*-benzylacetamide formation was not observed in the NMR spectra of the control solutions of unvaped CBN-OAc or of vaped CBN.

The amount of reacted ketene equivalents, as *N*-benzylacetamide, was determined by comparison of the peak area at 4.43 ppm to that of the internal standard and converting to mg ketene. The amount of ketene formed was 0.201 ± 0.0414 mg from a total of 103 mg of CBN-OAc, based on four replicates. Consumption of the entire 103 mg for each replicate required a total of four runs. Since 40 mg is more typical of an average dab size, ketene exposure under more realistic dabbing conditions is extrapolated to 0.078 mg.

In order to investigate the scope and generality of ketene formation from cannabinoid acetates, we studied $\Delta^8$-THC-OAc and CBD-$(OAc)_2$ under the same dabbing conditions used for CBN-OAc. The NMR spectra (Supporting Information) of the condensates collected in the impinger from $\Delta^8$-THC-OAc and CBD-$(OAc)_2$ display the characteristic doublet at 4.43 ppm for the benzylacetamide methylene, showing that ketene formation is general to dabbing the cannabinoid acetates under the conditions reported herein. The relative levels of ketene produced by $\Delta^8$-THC-OAc, CBD-$(OAc)_2$ and CBN-OAc were 1.0 : 13 : 3.6, respectively. To determine if ketene can form upon vape pen usage as well as under dabbing conditions, we vaped a commercial formulation containing $\Delta^8$-THC-OAc in a pre-filled, as-received vape pen cartridge. Evidence for ketene formation was also observed in the cartridge vape pen experiments, as shown in Figure 3.

**DISCUSSION**

**Ketene emission and exposure thresholds.** The results herein show ketene formation across different starting cannabinoid acetates and from both dabbing and e-cigarette cartridge vaping. There is a lack of peer-reviewed information concerning user preferences and vaping topography regarding cannabis

products. However, it is well-known that dabbing involves large inhalation volumes, approaching full lung capacity (~ 0.005 $m^3$). The 5.0 ppm threshold established by NIOSH for ketene exposure equates to 8.6 $mg/m^3$, using 1 ppm = 1.72 $mg/m^3$. In a 0.005 $m^3$ lung volume, the NIOSH threshold value is thus 0.043 mg ketene. The 0.078 mg yield of ketene obtained from a CBN-OAc dab is therefore above the NIOSH threshold. When the $\Delta^8$-THC-OAc sample was dabbed, the amount of ketene produced was lower (0.022 mg).

The actual levels of ketene from commercial products will typically be lower than what we have determined herein since other ingredients will likely also be present in the starting products. For example, the commercial sample purchased and studied for this work contained equimolar amounts of $\Delta^8$-THC-OAc and $\Delta^8$-THC, as well as terpenes. In addition, vaping with an e-cigarette does not involve large inhalation volumes relative to dabbing, and ketene inhaled from a vape pen will be relatively more dilute in the lungs. In addition, ketene also can react with water in the upper respiratory tract to form acetic acid prior to reaching the lungs.

Although realistic ketene emission levels may be lower than those reported under the conditions herein, chronic exposure is an additional potential concern, since toxicological data on chronic exposure to ketene is lacking. Moreover, available data on acute exposure to ketene has been described as dated and incomplete.[7] Ketene's most relevant toxicological property is its very high reactivity, as it readily chemically modifies biological molecules. [7] However, ketene does not survive long enough to be detected intact *in vivo*. Chemical and physical studies such as reported herein and by others[8, 14, 15 16-18] are thus required to elucidate sources of exposure.

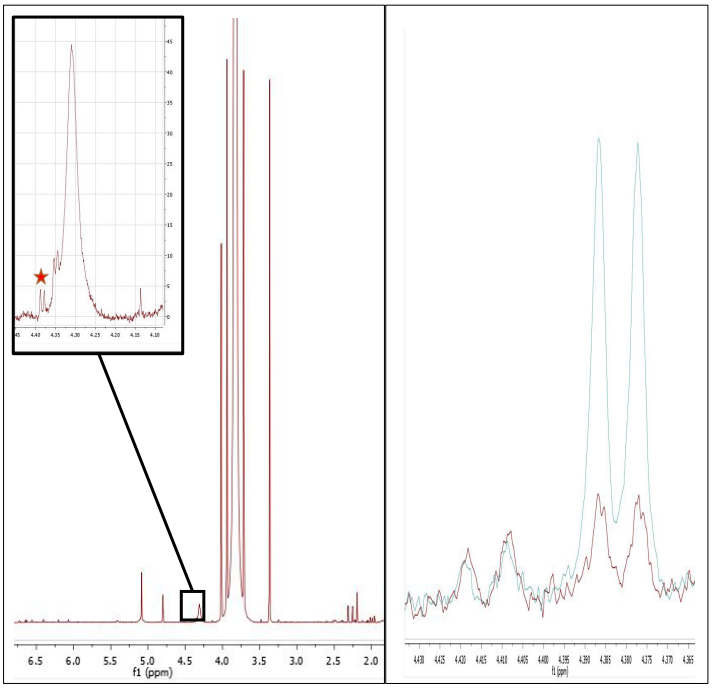



**Figure 3.** NMR spectra of the condensate obtained from vaping with a commercial cartridge containing $\Delta^8$-THC-OAc. Left: The *N*-benzylacetamide peak (starred, inset) indicating ketene formation. Right: The light blue spectrum corresponds to added *N*-benzylacetamide standard to the original NMR sample (red), confirming the peak assignment.

**Ketene formation temperature dependence.** A main question that arose after it was shown that vitamin E acetate produces ketene under vaping conditions is why ketene would form at relatively low vaping temperatures compared to temperatures used in its industrial production. The data herein shows that it forms from cannabinoid acetates with a dabbing surface temperature of 378 °C, as well as from a vape pen operating at a power level with the manufacturer's recommended conditions. Catalysis, viscosity, airflow, device quality and user behavior can each exacerbate toxicant formation, in addition to elevated temperature settings.[19] Importantly, the study herein thus validates the findings of Wu and O'Shea as well as others showing that vaping conditions can lead to ketene formation at apparently lower temperature settings than previously assumed. [8, 17] [14, 16]

**Study limitations.** This work describes a limited set of starting acetates, including three that have been purified and one commercial formulation. Despite the relatively few data points, the acetates, under either dabbing and e-cigarette vaping methods, consistently afforded observable ketene emissions. The reason for the varying levels of ketene observed from the three different dabbed samples is currently still unclear, although all were within the same order of magnitude, when accounting for the fact that CBD-$(OAc)_2$ has two reactive groups. Additional dabbing and vape pen experiments are planned, along with the investigation of other toxicant emissions associated with cannabis vaping.

**Conclusion.** The studies described herein show that ketene exposure can occur from vaping or dabbing cannabinoid acetates. This is not surprising, considering the fact that ketene was previously shown to form via a structurally-related phenyl acetate-containing compound, vitamin E acetate, under e-cigarette vaping conditions.[8] The ketene emission levels observed in the dabbing experiments were in range of the NIOSH IDLH value.[7] More studies are needed to understand the factors promoting ketene formation vaping cannabis and related products, along with in-depth profiling of the contents of cannabis oil condensates, and are underway in our laboratories.

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