Vitamin E Acetate in Bronchoalveolar-Lavage Fluid Associated with EVALI

B.C. Blount, M.P. Karwowski, P.G. Shields, M. Morel-Espinosa, L. Valentin-Blasini, M. Gardner, M. Braselton, C.R. Brosius, K.T. Caron, D. Chambers, J. Corstvet, E. Cowan, V.R. De Jesús, P. Espinosa, C. Fernandez, C. Holder, Z. Kuklenyik, J.D. Kusovschi, C. Newman, G.B. Reis, J. Rees, C. Reese, L. Silva, T. Seyler, M.-A. Song, C. Sosnoff, C.R. Spitzer, D. Tevis, L. Wang, C. Watson, M.D. Wewers, B. Xia, D.T. Heitkemper, I. Ghinai, J. Layden, P. Briss, B.A. King, L.J. Delaney, C.M. Jones, G.T. Baldwin, A. Patel, D. Meaney-Delman, D. Rose, V. Krishnasamy, J.R. Barr, J. Thomas, and J.L. Pirkle, for the Lung Injury Response Laboratory Working Group*

ABSTRACT

BACKGROUND

The causative agents for the current national outbreak of electronic-cigarette, or vaping, product use–associated lung injury (EVALI) have not been established. Detection of toxicants in bronchoalveolar-lavage (BAL) fluid from patients with EVALI can provide direct information on exposure within the lung.

METHODS

BAL fluids were collected from 51 patients with EVALI in 16 states and from 99 healthy participants who were part of an ongoing study of smoking involving nonsmokers, exclusive users of e-cigarettes or vaping products, and exclusive cigarette smokers that was initiated in 2015. Using the BAL fluid, we performed isotope dilution mass spectrometry to measure several priority toxicants: vitamin E acetate, plant oils, medium-chain triglyceride oil, coconut oil, petroleum distillates, and diluent terpenes.

RESULTS

State and local health departments assigned EVALI case status as confirmed for 25 patients and as probable for 26 patients. Vitamin E acetate was identified in BAL fluid obtained from 48 of 51 case patients (94%) in 16 states but not in such fluid obtained from the healthy comparator group. No other priority toxicants were found in BAL fluid from the case patients or the comparator group, except for coconut oil and limonene, which were found in 1 patient each. Among the case patients for whom laboratory or epidemiologic data were available, 47 of 50 (94%) had detectable tetrahydrocannabinol (THC) or its metabolites in BAL fluid or had reported vaping THC products in the 90 days before the onset of illness. Nicotine or its metabolites were detected in 30 of 47 of the case patients (64%).

CONCLUSIONS

Vitamin E acetate was associated with EVALI in a convenience sample of 51 patients in 16 states across the United States. (Funded by the National Cancer Institute and others.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Blount at the Centers for Disease Control and Prevention, Mailstop F47, 4770 Buford Hwy. NE, Atlanta, GA 30341, or at bkb3@cdc.gov.

*A list of the members of the Lung Injury Response Laboratory Working Group is provided in the Supplementary Appendix, available at NEJM.org.

Drs. Blount and Karwowski contributed equally to this article.

697

AS OF DECEMBER 12, 2019, THE ONGOING national outbreak of electronic-cigarette, or vaping, product use–associated lung injury (EVALI) has affected more than 2400 hospitalized patients across all 50 states, the District of Columbia, the U.S. Virgin Islands, and Puerto Rico. In addition, 52 deaths had been reported in 25 states and the District of Columbia.[1] Most patients with EVALI (78%) have been younger than 35 years of age and have reported a gradual onset of symptoms — including respiratory (95%), constitutional (85%), and gastrointestinal (77%) symptoms — over days to weeks.[2] Nearly half the patients (47%) required intensive care to treat respiratory failure.[2] Most patients have reported the use of e-cigarette products containing tetrahydrocannabinol (THC).[3] Radiographic findings may not be apparent on presentation, but such patients commonly are found to have basilar-predominant consolidation and ground-glass opacity.[4] Histopathological features of acute lung injury have been described, including diffuse alveolar damage, acute fibrinous pneumonitis, and organizing pneumonia.[5]

EVALI cases were first reported to the Centers for Disease Control and Prevention (CDC) in August 2019 and rapidly increased in number thereafter, which suggests new or increased exposure to one or more toxicants from the use of e-cigarette products. The initial laboratory strategy to help determine the cause of EVALI was to analyze product fluids associated with case patients to detect toxicants in these products that could account for the lung injury. In September, the New York State Department of Health Wadsworth Center Laboratory reported results for 34 persons who used THC-containing e-cigarette products and had pulmonary illness.[6] Many of these patients had used multiple products; however, each one had used at least one THC-containing product that tested positive for vitamin E acetate, a chemical that is sometimes used as a thickening agent in illicit THC-containing products. The Food and Drug Administration (FDA) also analyzed product fluids for vitamin E acetate, diluents, metals, and other selected chemicals. Patients with EVALI have commonly used multiple tobacco and cannabis products.[7] FDA investigators analyzed multiple product samples for each patient, some of which may or may not have contained a causative agent. Among case-associated THC-containing product samples, in-

vestigators found vitamin E acetate in 49% of the samples and at least one other potential toxicant (e.g., medium-chain triglycerides) in 24% of the samples.[8] Furthermore, trade websites[9-11] have reported the addition of various diluents (e.g., vitamin E acetate and medium-chain triglycerides) to THC-containing products to enhance their quality and appearance, provide desirable aroma or taste, and lower product cost.

As an expansion of the laboratory strategy for the lung-injury response, the CDC, in consultation with the FDA, began testing for harmful substances in bronchoalveolar-lavage (BAL) fluid obtained from patients with EVALI. The CDC put primary emphasis on the analysis of BAL fluid because this approach could detect harmful substances at the presumed site of the lung injury. In collaboration with state health departments, the CDC obtained BAL fluid samples from 29 patients with EVALI in 10 states, with analysis showing the presence of vitamin E acetate in all 29 samples.[12] This study extends that work by analyzing samples from 51 patients with EVALI in 16 states along with samples obtained from 99 healthy participants in a smoking-related study that included never smokers, exclusive e-cigarette users, and exclusive cigarette smokers.

METHODS

CASE DEFINITIONS

All case-associated BAL fluid samples were obtained from patients who met EVALI case definitions of the CDC, as determined by state or local health officials (Table 1).[13]

BIOSPECIMEN COLLECTION FROM CASE AND COMPARATOR PARTICIPANTS
EVALI Case Patients

BAL fluid was obtained by clinical teams caring for hospitalized patients. The decision to obtain BAL fluid was made by the clinical team on a case-by-case basis to guide clinical management decisions. The CDC requested the assistance of state health departments in acquiring BAL fluid samples. If a patient qualified as having a probable or confirmed diagnosis of EVALI, then BAL fluid was accepted by the CDC for analysis. No other inclusion or exclusion criteria were applied.

The small volumes of BAL fluid in some samples required prioritizing analyses. Vitamin E acetate was prioritized first, followed by dipal-

mitoylphosphatidylcholine (DPPC), cannabinoids, and then other toxicants. Insufficient clinical data were available to estimate the time between the last use of an e-cigarette product and the bronchoscopy to obtain BAL fluid. Sample collection was conducted according to the routine clinical processes at each institution and was not standardized. Samples were refrigerated or frozen after collection and shipped to the CDC on dry ice. Human-subjects research review was conducted by the CDC, which determined that this information collection did not meet the regulatory definition of research under 45 CFR 46.102(d) and was therefore determined to be a nonresearch public health response activity.

Healthy Comparator Group

BAL fluid had been previously obtained from 99 participants that included nonusers, current exclusive users of nicotine-containing e-cigarette products, and current exclusive cigarette smokers recruited through local media and participant registries at Ohio State University[14,15] as part of a tobacco-product study unrelated to the ongoing EVALI investigation. The participants were between the ages of 21 and 45 years and had no major clinical illness or increased risk from bronchoscopy. Inclusion and exclusion criteria for the 52 nonusers, the 18 exclusive users of nicotine-containing e-cigarette products, and the 29 exclusive cigarette smokers have been reported previously.[15] The negative smoking status for both the nonusers and users of e-cigarette products was confirmed biochemically by urinary anatabine testing.[15] After collection, BAL fluids were transported to the laboratory on wet ice and processed within 30 minutes by centrifugation; cell pellets and supernatant were immediately placed in storage at −80°C.

All the participants provided written informed consent before participation. The tobacco-product study involving healthy participants was approved by the institutional review board at Ohio State University.[16]

LABORATORY ANALYSIS

Investigators at the CDC established a list of priority toxicants to measure in BAL fluid: vitamin E acetate, plant oils (identified by measuring long-chain triglycerides), medium-chain triglyceride oil, coconut oil (identified by measuring medium-chain triglycerides), petroleum distillates,

Table 1. Case Definitions for Patients with EVALI.*

Confirmed Case	Probable Case
Use of e-cigarette ("vaping") or dabbing† during the 90 days before symptom onset *AND*	Use of e-cigarette ("vaping") or dabbing† during the 90 days before symptom onset *AND*
Presence of pulmonary infiltrate, such as opacities, on chest radiography or ground-glass opacities on chest computed tomography *AND*	Presence of pulmonary infiltrate, such as opacities, on chest radiography or ground-glass opacities on chest computed tomography *AND*
A negative respiratory viral panel *AND* A negative influenza PCR or rapid test, if local epidemiology supports influenza testing *AND* Negative results on testing for all other clinically indicated respiratory infectious diseases (e.g., urine antigen for *Streptococcus pneumoniae* and legionella species, sputum culture in the presence of productive cough, bronchoalveolar-lavage culture if performed, blood culture, and HIV-related opportunistic respiratory infections if appropriate) *AND*	Presence of infection identified on culture or PCR, but clinical team determines that this infection is not the sole cause of the underlying lung injury *OR* the minimum criteria to rule out pulmonary infection are not met (or testing not performed) and clinical team determines that this infection is not the sole cause of the underlying lung injury *AND*
No evidence in medical record of alternative plausible diagnoses (e.g., cardiac, rheumatologic, or neoplastic process)	No evidence in medical record of alternative plausible diagnoses (e.g., cardiac, rheumatologic, or neoplastic process).

* EVALI denotes electronic-cigarette, or vaping, product use–associated lung injury, HIV human immunodeficiency virus, and PCR polymerase chain reaction.
† This criterion is defined as the use of an electronic device (e.g., electronic nicotine-delivery system, e-cigarette, vaporizer, or other device) or dabbing to inhale substances (e.g., nicotine, marijuana, tetrahydrocannabinol [THC], THC concentrates, cannabidiol, synthetic cannabinoids, flavorings, and other substances).

and diluent terpenes (including limonene). For these toxicants in BAL fluid, the CDC developed and validated analytic methods that were evaluated for accuracy, precision, analytic sensitivity, and analytic specificity. These methods used isotope dilution mass spectrometry and provided results that met or exceeded the quality criteria of the Clinical Laboratory Improvement Amendments.

The analytic limit of detection (LOD) was calculated on the basis of repeated measures to estimate the standard deviation at zero concentration.[17] Results higher than the LOD are reported as positive, and results lower than the LOD are reported as negative. The methods to measure each of the potential toxicants of concern — along with THC and nicotine parent compounds and metabolites and DPPC — are

Table 2. Characteristics of EVALI Case Patients and Healthy Comparators.*

	EVALI Case Patients (N=51)	Healthy Comparators			
		Nonusers (N=52)	E-Cigarette Users (N=18)	Cigarette Smokers (N=29)	All Comparators (N=99)
Median age (range) — yr	23 (16–67)	25 (21–37)	27 (21–30)	26 (21–44)	26 (21–44)
Male sex — no. (%)	35 (69)	19 (37)	12 (67)	22 (76)	53 (54)
Self-reported vaping — no./ total no. (%)					
Nicotine products only	7/43 (16)	0/52	18/18 (100)	29/29 (100)	47/99 (47)
THC products only	11/43 (26)	0/52	0/18	0/29	0/99
Dual use of nicotine and THC products	22/43 (51)	0/52	0/18	0/29	0/99
Urinary carboxy-THC level ≥3.0 ng/ml — no./ total no. (%)	NA	4/42 (10)	4/17 (24)	13/23 (57)	21/82 (26)

* NA denotes not analyzed.

described in Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org.[18-20] All case and comparator samples of BAL fluid had detectable levels of DPPC, the main phospholipid of lung surfactant; DPPC was used as a marker to indicate that the BAL fluid contained pulmonary epithelial-lining fluid and thus was considered to be an adequate sample for testing.

DATA ANALYSIS

Results are presented as percentages of persons in whom BAL fluid samples tested positive for the measured toxicant or metabolite biomarker. Because of the limited volume of BAL fluid, results for all analytes were not available for all participants. Since the analysis of vitamin E acetate was prioritized, results regarding vitamin E acetate are reported for all the participants. Denominators reflect the actual number of participants for whom samples were tested for each analyte.

RESULTS

From August through December 2019, public health laboratories and health departments in 16 states provided BAL fluid samples obtained from 51 patients, including those in Arizona (2), Arkansas (2), California (4), Connecticut (1), Hawaii (1), Illinois (12), Indiana (1), Maryland (4), Michigan (2), Minnesota (5), Missouri (1), North Carolina (2), Texas (2), Utah (3), West Virginia (1), and Wisconsin (8). BAL fluid was acquired from 3 patients with EVALI who later died.

The age and sex of the 51 patients for whom BAL fluid was available were consistent with national data regarding patients with EVALI.[7] The median age of the patients was 23 years, and 69% were male (Table 2). Among the patients for whom information was available, 77% reported the use of THC-containing products, 67% reported the use of nicotine-containing products, and 51% reported the use of both types of products. Health departments assigned and reported case status for all patients; of these patients, 25 had been classified as having confirmed EVALI and 26 as having probable EVALI.

Samples from healthy study participants were collected from 2016 through 2019, with 90 of the 99 samples obtained before 2019. The mean (±SD) age in years was 25.4±3.6 for nonusers, 26.5±2.7 for exclusive e-cigarette users, and 27.3±4.9 for exclusive cigarette smokers. Men made up 37%, 67%, and 76%, respectively, of the subgroups. Among the healthy participants, urine THC biomarkers were positive in 25.6%, whereas BAL fluid THC biomarkers were positive in 5.1%. The higher sensitivity of urine than of BAL fluid for the detection of THC biomarkers has been reported previously.[21] The prevalence of positive urine THC biomarkers was highest among the participants who reported exclusive cigarette smoking (13 of 23 [57%]). Nicotine or its metabolites were detected in 52% of BAL fluids from the healthy participants. All

Table 3. Frequency of Detection of Priority Toxicants in EVALI Case Patients and in Healthy Comparators.*

Toxicant	EVALI Case Patients (N=51)	Healthy Comparators			
		Nonusers (N=52)	E-Cigarette Users (N=18)	Cigarette Smokers (N=29)	All Comparators (N=99)
		number/total number (percent)			
Vitamin E acetate	48/51 (94)	0/52	0/18	0/29	0/99
Medium-chain tri-glyceride oil	0/49	0/34	0/11	0/18	0/63
Coconut oil	1/48 (2)	0/34	0/11	0/18	0/63
Plant oil	0/49	0/34	0/11	0/17	0/62
Squalane	0/38	0/52	0/17	0/29	0/98
Squalene	0/38	0/52	0/17	0/29	0/98
α-Pinene	0/39	0/52	0/17	0/28	0/97
β-Pinene	0/39	0/52	0/17	0/28	0/97
3-Carene	0/39	0/52	0/17	0/28	0/97
Limonene	1/39 (3)	0/52	0/17	0/28	0/97
Petroleum distillates	0/12	0/52	0/17	0/29	0/98

* The listed toxicants were detected in bronchoalveolar-lavage fluid obtained from 51 patients with EVALI in 16 states from August through December 2019 and in 99 healthy comparators.

the BAL fluid samples from this group were negative for all priority toxicants: vitamin E acetate, plant oils, medium-chain triglyceride oil, coconut oil, petroleum distillates, and diluent terpenes.

Among the patients with EVALI, vitamin E acetate was detected in BAL fluid samples from 48 of 51 (94%) (Table 3). In 1 patient, BAL fluid had detectable levels of coconut oil in addition to vitamin E acetate; in another patient, BAL fluid contained limonene (a diluent terpene) with no detectable vitamin E acetate or THC. Samples from all the other patients were negative for the other priority toxicants: plant oils, medium-chain triglyceride oil, coconut oil, petroleum distillates, and diluent terpenes. Table 4 provides a clinical description of the 3 patients with EVALI who had no detectable vitamin E acetate; all of these 3 patients were classified as having probable cases. THC or its metabolites were detected in BAL fluid samples from 40 of 47 patients (85%) with EVALI. A total of 9 of 11 patients who reported no use of THC-containing e-cigarette products in the 90 days before the onset of illness had detectable THC or its metabolites in their BAL fluid. Among the patients who had available laboratory data or who reported product use, 47 of 50 (94%) had detectable THC

or its metabolites in BAL fluid or reported vaping THC products in the 90 days before the onset of illness. Nicotine or its metabolites were detected in 30 of 47 (64%) BAL fluid samples from case patients.

DISCUSSION

In our study, the detection of vitamin E acetate in BAL fluid in most of the patients with EVALI provides evidence that e-cigarette, or vaping, products can deliver vitamin E acetate to respiratory epithelial-lining fluid, the presumed site of injury in the lung. In this convenience sample, the finding that 48 of 51 patients with lung injury had vitamin E acetate in their BAL fluid is noteworthy. By contrast, vitamin E acetate was not detected in any BAL fluid collected from 99 healthy participants, including 18 e-cigarette users. In addition, the absence of other toxicants (plant oils, medium-chain triglyceride oil, coconut oil, petroleum distillates, and diluent terpenes) in BAL fluids from nearly all patients with EVALI provides evidence against the role of any of these toxicants as a primary cause of EVALI. Whether limonene or coconut oil (found in the BAL fluid of 1 patient each) may have some toxicologic effect is unclear.

Table 4. Exposure History and Clinical Characteristics of Three Patients with Probable Diagnosis of EVALI without Evidence of Vitamin E Acetate in BAL Fluid.*

Variable	Patient 1	Patient 2	Patient 3
Patient-reported exposure history	Reported vaping nicotine products, denied vaping THC products	Reported daily vaping of flavored nicotine products obtained from vape shop starting 1 mo before admission, denied vaping THC products	Incomplete interview with the patient; vaping materials found at the scene by first responders; patient later reported heavy alcohol use immediately before illness
Symptoms	Respiratory, gastrointestinal	Constitutional, respiratory	Constitutional, respiratory
Medical history	Negative for chronic respiratory disease and heart disease	Negative for chronic respiratory disease and heart disease	Negative for chronic respiratory disease and heart disease
Presentation	Found unresponsive; on arrival at emergency department, hemoptysis and cyanosis; was intubated and admitted	Presented to urgent care on day of illness (DOI) 1 and to emergency department on DOI 3 and 5, when he was admitted	Found unresponsive; on arrival at emergency department, severe respiratory failure; was intubated and admitted
Admitted to intensive care unit	Yes	No	Yes
Respiratory support	Mechanical ventilation	None	Mechanical ventilation
Radiologic assessment	Hazy opacities predominantly in left lung on radiography; no CT	Bilateral patchy opacities on radiography; diffuse bilateral nodular opacities with surrounding micronodular and ground-glass opacities on CT	Bilateral infiltrates and opacities on radiography and CT
Infectious diseases workup	Blood cultures negative; methicillin-susceptible Staphylococcus aureus (interpreted by clinical team as a contaminant) in BAL fluid	Serologic analysis on admission indeterminate for coccidioides species, follow-up IgM and IgG by immunodiffusion were positive; BAL cell count, 31% eosinophils; negative results on respiratory viral panel, influenza PCR, blood cultures, legionella urinary antigen, Streptococcus pneumoniae urinary antigen, Mycoplasma pneumoniae PCR, cytomegalovirus PCR, pneumocystis antigen, fungal stain and culture, AFB smear, and mycobacterial culture	Negative results on respiratory viral panel, influenza testing, and blood cultures; methicillin-susceptible S. aureus (interpreted by clinical team as a contaminant) in tracheal-aspirate culture
Treatment	Glucocorticoids and antibiotics	Glucocorticoids, antibiotics, and antifungal agents	Glucocorticoids and antibiotics
Disposition	Discharged 3 days after admission	Discharged 3 days after admission	Discharged 13 days after admission
Discharge diagnoses	Unintentional multidrug overdose with benzodiazepines and oxycodone, active nicotinism with vaping, with suspected likely vaping-induced lung injury	Acute eosinophilic pneumonia, initially attributed to EVALI; after discharge, positive results on coccidioides serologic analysis prompted updating of diagnosis to coccidioidomycosis with or without EVALI	Acute hypoxic respiratory failure caused by vaping-associated lung injury, methicillin-susceptible S. aureus pneumonia, acute respiratory distress syndrome, distributive shock

* AFB denotes acid-fast bacilli, BAL bronchoalveolar lavage, and CT computed tomography.

Vitamin E acetate was not detected in the BAL fluid obtained from three patients with probable EVALI. Since EVALI is a diagnosis of exclusion for which there is no confirmatory diagnostic test, we could not confirm case status for these three patients. The EVALI case definition is intentionally sensitive, which raises the likelihood that a patient's illness could be misattributed to EVALI. The alternative diagnoses that are listed in Table 4 could reasonably explain the lung injury in these patients. Of note, the two patients who were successfully interviewed by public health officials denied vaping THC products. Reliable information on the time interval from the last use of an e-cigarette product until bronchoscopy is not available, so we cannot assess the likelihood that vitamin E acetate may have already been cleared from the lung in these patients. The detection of vitamin E acetate and other oils can be impaired by inadequate mixing of BAL fluid. Because BAL fluid was collected and processed in the local hospital setting according to its own protocols, we could not confirm that samples were adequately mixed. Patient 2 (as described in Table 4) reported the daily use of flavored nicotine products and had positive results for limonene (which is found in flavored nicotine solutions) in BAL fluid. Whether limonene has a toxicologic effect is unclear.[22,23]

Vitamin E acetate is commonly used as a dietary supplement and in skin creams. It is common in multivitamins and is enzymatically cleaved to vitamin E during absorption.[24,25] Although the ingestion and dermal application of vitamin E acetate have not generally been associated with adverse health effects, the safety of inhaling vitamin E acetate has received little attention. Vitamin E acetate is the ester of vitamin E (α-tocopherol) and acetic acid. The structure shows a long aliphatic tail that can penetrate a layer of surfactant to align the molecule in parallel with phospholipids[26] (Fig. S1). Phosphatidylcholines undergo transition from a gel to a liquid crystalline phase when exposed to increasing amounts of tocopherols, such as vitamin E acetate.[26,27] Transitioning to a liquid crystalline phase[25] would cause the surfactant to lose its ability to maintain the surface tension that is necessary to support respiration in the lung,[26,28,29] thus providing a possible mechanism by which vitamin E acetate could cause respiratory dysfunction.

Another potential harmful effect of vitamin E acetate that may contribute to lung injury occurs when it is heated in e-cigarette products. Heating vitamin E acetate in these devices may create ketene by splitting off the acetate group from some or all of the vitamin E acetate.[30] Ketene is a reactive compound that has the potential to be a lung irritant, depending on concentration. The CDC is currently examining ways to quantify ketene and its effects in BAL fluid.

Data that have been reported to date indicate that vitamin E acetate in the supply of THC-containing products and use among patients with EVALI aligns with the timing of the 2019 EVALI outbreak. In Minnesota, 10 of 10 products seized by law enforcement during 2018, before the EVALI outbreak, did not contain vitamin E acetate, whereas 20 of 20 THC-containing products seized by law enforcement during September 2019, at the peak of the outbreak, contained vitamin E acetate.[31] This finding is consistent with laboratory measurements and trade websites, which suggests that the addition of vitamin E acetate to product fluid began to appear in the illicit market in late 2018 or early 2019 and gained popularity in 2019.

Pure THC oil has a viscosity like that of vitamin E acetate. Cutting THC oil with vitamin E acetate has been reported to be common in the illicit market.[9-11] The FDA reports that most case-associated THC product fluids contain vitamin E acetate, at an average concentration of 50% by weight, ranging from 23 to 88%.[8] By contrast, the FDA detected no vitamin E acetate in 197 case-associated nicotine products analyzed to date. The viscosity of vitamin E acetate makes it undesirable as an additive to nicotine solutions; the propylene glycol and vegetable glycerin in nicotine solutions create a fluid with a much lower viscosity than that of vitamin E acetate.

Additional studies (including studies in animals) are in progress to examine the respiratory effects of inhaling aerosolized vitamin E acetate and will provide information on whether vitamin E acetate in isolation causes lung injury.[32] Research analyzing the aerosol and gases generated by case-associated product fluids is ongoing.

This study has several limitations. First, it is possible that vitamin E acetate may be a marker for exposure to alternative toxicants. Such an alternative toxicant would need to meet at least three criteria: correlate with the presence of vitamin E acetate in BAL fluid, be in widespread use

across the United States, and have been added to THC product fluids in 2019 or substantially increased in concentration in 2019 to match the timing of the outbreak. We have not been able to identify a toxicant that meets these three requirements but continue to study this possibility. Second, the contribution to injury of aerosol constituents formed from the heating of vitamin E acetate, especially at higher voltages, requires further examination. Until aerosolized constituents are better characterized, it is possible that one or more of them could act alone or synergistically with other compounds such as vitamin E acetate to increase the risk of EVALI. Third, sample collection was performed as part of routine clinical care and thus was not standardized. Fourth, the timing and burden of exposure to the potential toxicants in relation to the BAL sample acquisition could not be assessed. Finally, this study reports data for case patients and healthy comparators from an independent cross-sectional study, which limits insights because samples were collected at a single point in time, and the possibility of unknown confounding remains.

Vitamin E acetate may play a role in EVALI, a conclusion supported by the high detection rate of vitamin E acetate in BAL fluid from case patients who live in 16 states across the United States and the absence of vitamin E acetate in samples obtained from a healthy comparison group, by the absence of other priority toxicants in nearly all BAL fluid samples from case patients, by a biologically plausible mechanism for lung injury associated with vitamin E acetate, and by the temporal alignment between EVALI and the use of vitamin E acetate in the illicit marketplace. Results from studies in animals can provide information on whether exposure to vitamin E acetate alone can directly cause the lung injury seen in patients with EVALI.

APPENDIX

The authors' full names and academic degrees are as follows: Benjamin C. Blount, Ph.D., Mateusz P. Karwowski, M.D., M.P.H., Peter G. Shields, M.D., Maria Morel-Espinosa, Ph.D., Liza Valentin-Blasini, Ph.D., Michael Gardner, M.S., Martha Braselton, B.S., Christina R. Brosius, M.P.H., Kevin T. Caron, B.S., David Chambers, Ph.D., Joseph Corstvet, B.S., Elizabeth Cowan, Ph.D., Víctor R. De Jesús, Ph.D., Paul Espinosa, B.S., Carolina Fernandez, M.P.H., Cory Holder, M.S., Zsuzsanna Kuklenyik, Ph.D., Jennifer D. Kusovschi, B.S., Cody Newman, B.S., Gregory B. Reis, B.S., Jon Rees, Ph.D., Chris Reese, B.S., Lalith Silva, Ph.D., Tiffany Seyler, Ph.D., Min-Ae Song, Ph.D., Connie Sosnoff, M.A., Carleen R. Spitzer, M.D., Denise Tevis, M.S., Lanqing Wang, Ph.D., Cliff Watson, Ph.D., Mark D. Wewers, M.D., Baoyun Xia, Ph.D., Douglas T. Heitkemper, Ph.D., Isaac Ghinai, M.B., B.S., Jennifer Layden, M.D., Ph.D., Peter Briss, M.D., Brian A. King, Ph.D., Lisa J. Delaney, M.S., Christopher M. Jones, Pharm.D., Dr.P.H., Grant T. Baldwin, Ph.D., Anita Patel, Pharm.D., Dana Meaney-Delman, M.D., Dale Rose, Ph.D., Vikram Krishnasamy, M.D., John R. Barr, Ph.D., Jerry Thomas, M.D., and James L. Pirkle, M.D., Ph.D.

The authors' affiliations are as follows: the Division of Laboratory Sciences, National Center for Environmental Health (B.C.B., M.P.K., M.M.-E., L.V.-B., M.G., M.B., C.R.B., K.T.C., D.C., J.C., E.C., V.R.D.J., P.E., C.F., C.H., Z.K., J.D.K., C.N., G.B.R., J.R., C.R., L.S., T.S., C.S., D.T., L.W., C.W., B.X., J.R.B., J.T., J.L.P.), Epidemic Intelligence Service, Center for Surveillance, Epidemiology, and Laboratory Sciences (I.G.), the Office of the Director (P.B.) and the Office on Smoking and Health (B.A.K.), National Center for Chronic Disease Prevention and Health Promotion, the Office of the Director, National Institute for Occupational Safety and Health (L.J.D.), the Office of Strategy and Innovation (C.M.J.) and the Division of Overdose Prevention (G.T.B.), National Center for Injury Prevention and Control, the Office of the Director, National Center for Immunization and Respiratory Diseases (A.P.), the Division of Birth Defects and Infant Disorders, National Center on Birth Defects and Developmental Disabilities (D.M.D.), the Division of Preparedness and Emerging Infections, National Center for Emerging and Zoonotic Infectious Diseases (D.R.), and the Office of the Director, National Center for Injury Prevention and Control (V.K.) — all at the Centers for Disease Control and Prevention, Atlanta; the Comprehensive Cancer Center, Ohio State University and James Cancer Hospital (P.G.S.), and the Division of Environmental Health Science, College of Public Health (M.-A.S.), and Pulmonary and Critical Care Medicine, Department of Internal Medicine (C.R.S., M.D.W.), Ohio State University, Columbus; the Food and Drug Administration, Silver Spring, MD (D.T.H.); and the Illinois Department of Public Health, Chicago (I.G., J.L.).

REFERENCES

1. Centers for Disease Control and Prevention. Outbreak of lung injury associated with e-cigarette use, or vaping. December 12, 2019 (https://www.cdc.gov/tobacco/basic_information/e-cigarettes/severe-lung-disease.html?s_cid=osh-stu-home-spotlight-006).
2. Siegel DA, Jatlaoui TC, Koumans EH, et al. Update: interim guidance for health care providers evaluating and caring for patients with suspected e-cigarette, or vaping, product use associated lung Injury — United States, October 2019. Am J Transplant 2019;19:3420-8.
3. Lozier MJ, Wallace B, Anderson K, et al.

Update: demographic, product, and substance-use characteristics of hospitalized patients in a nationwide outbreak of e-cigarette, or vaping, product use-associated lung injuries — United States, December 2019. MMWR Morb Mortal Wkly Rep 2019;68:1142-8.

4. Henry TS, Kligerman SJ, Raptis CA, Mann H, Sechrist JW, Kanne JP. Imaging findings of vaping-associated lung injury. AJR Am J Roentgenol 2019 October 8 (Epub ahead of print).

5. Butt YM, Smith ML, Tazelaar HD, et al. Pathology of vaping-associated lung injury. N Engl J Med 2019;381:1780-1.

6. New York State Department of Health. New York State Department of Health announces update on investigation into vaping-associated pulmonary illnesses: department warns against use of black market vaping products: lab test results show high levels of vitamin E acetate, now focus of investigation. September 5, 2019 (https://www.health.ny.gov/press/releases/2019/2019-09-05_vaping.htm).

7. Moritz ED, Zapata LB, Lekiachvili A, et al. Update: characteristics of patients in a national outbreak of e-cigarette, or vaping, product use-associated lung injuries — United States, October 2019. MMWR Morb Mortal Wkly Rep 2019;68:985-9.

8. Food and Drug Administration. Lung illnesses associated with use of vaping products: information for the public, FDA actions, and recommendations. October 4, 2019 (https://www.fda.gov/news-events/public-health-focus/lung-illnesses-associated-use-vaping-products#Analysis).

9. Downs D. Vape pen lung injury: here's what you need to know. December 12, 2019 (https://www.leafly.com/news/health/vape-pen-lung-disease-advice-consumers).

10. Downs D, Howard D, Barcott B. Journey of a tainted vape cartridge: from China's labs to your lungs. Leafly. September 24, 2019 (https://www.leafly.com/news/politics/vape-pen-injury-supply-chain-investigation-leafly).

11. Eisenberg Z, Moy D, Lam V, Cheng C, Richard J, Burack B. Contaminant analysis of illicit vs regulated market extracts. San Francisco: Anresco Laboratories, October 26, 2019 (https://cannabis.anresco.com/analysis-of-illicit-vs-regulated-market-extracts).

12. Blount BC, Karwowski MP, Morel-Espinosa M, et al. Evaluation of bronchoalveolar lavage fluid from patients in an out-break of e-cigarette, or vaping, product use–associated lung injury — 10 states, August–October 2019. MMWR Morb Mortal Wkly Rep 2019;68:1040-1.

13. Centers for Disease Control and Prevention. Smoking and tobacco use: for state and local health departments. December 12, 2019 (https://www.cdc.gov/tobacco/basic_information/e-cigarettes/severe-lung-disease/health-departments/index.html#primary-case-def).

14. Tsai M, Song MA, McAndrew C, et al. Electronic versus combustible cigarette effects on inflammasome component release into human lung. Am J Respir Crit Care Med 2019;199:922-5.

15. Song MA, Freudenheim JL, Brasky TM, et al. Biomarkers of exposure and effect in the lungs of smokers, non-smokers and electronic cigarette users. Cancer Epidemiol Biomarkers Prev 2019 December 17 (Epub ahead of print).

16. Ohio State University Institutional Review Board. Effects of electronic cigarette use on the lungs. OSU-2015C0088; NCT02596685. 2019 (https://clinicaltrials.gov/ct2/show/NCT02596685?term=NCT02596685&draw=1&rank=1).

17. Taylor JK. Quality assurance of chemical measurements. New York: Lewis Publishers, 1987.

18. Cao Z, Schmitt TC, Varma V, Sloper D, Beger RD, Sun J. Evaluation of the performance of Lipidyzer platform and its application in the lipidomics analysis in mouse heart and liver. J Proteome Res 2019 July 26 (Epub ahead of print).

19. Orsavova J, Misurcova L, Ambrozova JV, Vicha R, Mlcek J. Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. Int J Mol Sci 2015;16:12871-90.

20. Chambers DM, Blount BC, McElprang DO, Waterhouse MG, Morrow JC. Picogram measurement of volatile n-alkanes (n-hexane through n-dodecane) in blood using solid-phase microextraction to assess nonoccupational petroleum-based fuel exposure. Anal Chem 2008;80:4666-74.

21. Rotolo MC, Pellegrini M, Martucci P, et al. Cannabinoids determination in bronchoalveolar lavages of cannabis smokers with lung disease. Clin Chem Lab Med 2019;57:498-503.

22. National Center for Biotechnology In-formation. PubChem database: limonene, CID=22311 (https://pubchem.ncbi.nlm.nih.gov/compound/Limonene).

23. Larsen ST, Hougaard KS, Hammer M, et al. Effects of R-(+)- and S-(-)-limonene on the respiratory tract in mice. Hum Exp Toxicol 2000;19:457-66.

24. Desmarchelier C, Tourniaire F, Prévé-raud DP, et al. The distribution and relative hydrolysis of tocopheryl acetate in the different matrices coexisting in the lumen of the small intestine during digestion could explain its low bioavailability. Mol Nutr Food Res 2013;57:1237-45.

25. Reboul E. Vitamin E bioavailability: mechanisms of intestinal absorption in the spotlight. Antioxidants (Basel) 2017; 6(4):E95.

26. Kamal MA, Raghunathan VA. Modulated phases of phospholipid bilayers induced by tocopherols. Biochim Biophys Acta 2012;1818:2486-93.

27. Massey JB, She HS, Pownall HJ. Interaction of vitamin E with saturated phospholipid bilayers. Biochem Biophys Res Commun 1982;106:842-7.

28. Zuo YY, Veldhuizen RA, Neumann AW, Petersen NO, Possmayer F. Current perspectives in pulmonary surfactant — inhibition, enhancement and evaluation. Biochim Biophys Acta 2008;1778:1947-77.

29. Casals C, Cañadas O. Role of lipid ordered/disordered phase coexistence in pulmonary surfactant function. Biochim Biophys Acta 2012;1818:2550-62.

30. Wu D, O'Shea DF. Potential for release of pulmonary toxic ketene from vaping pyrolysis of vitamin E acetate. ChemRxiv. December 6, 2019 (https://chemrxiv.org/articles/Potential_for_Release_of_Pulmonary_Toxic_Ketene_from_Vaping_Pyrolysis_of_Vitamin_E_Acetate/10058168).

31. Taylor J, Wiens T, Peterson J, et al. Characteristics of e-cigarette, or vaping, products used by patients with associated lung injury and products seized by law enforcement — Minnesota, 2018 and 2019. MMWR Morb Mortal Wkly Rep 2019;68:1096-100.

32. National Institutes of Health. Notice of Special Interest (NOSI): availability of administrative and revision supplements to expand vaping research and understand EVALI — Notice Number: NOT-HL-19-724. November 6, 2019 (https://grants.nih.gov/grants/guide/notice-files/NOT-HL-19-724.html).

Copyright © 2019 Massachusetts Medical Society.

ARTICLE METRICS NOW AVAILABLE

Visit the article page at NEJM.org and click on Metrics to view comprehensive and cumulative article metrics compiled from multiple sources, including Altmetrics. NEJM.org/about-nejm/article-metrics.

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Vitamin E Acetate in Bronchoalveolar Lavage Fluid Associated with EVALI

Benjamin C. Blount, PhD[1]*; Mateusz P. Karwowski, MD, MPH[1]*; Peter G. Shields, MD[2] et al.

Table of Contents

Lung Injury Response Laboratory Working Group:
Kathleen Attfield, ScD[1]; Robert J. Blount, MD2; Theodore M. Brasky, PhD[3]; Bobby N. Brewer, PhD[4]; Emily M. Carlson, MPH[5]; Kevin Chatham-Stephens, MD[6]; Sascha Ellington, PhD[7]; Mary E. Evans, MD[8]; Jeff Fowles, PhD[1]; Jennifer Freed, MPH[9]; Jo L. Fruedenheim, PhD[10]; Tiana Galindo[5]; Macarena Garcia, DrPH[11]; Althea Grant, PhD[12]; Donald Hayes, MD[13]; Amy Heinzerling, MD, MPH[1]; Tara C. Jatlaoui, MD[7]; Rudolph Johnson, PhD[14]; Joseph G. Lisko, MS[14]; Matthew J. Lozier, PhD[15]; Ruth Lynfield, MD[16]; Joseph P. McElroy, PhD[3]; Paul Melstrom, PhD[17]; Christina Mikosz, MD[18]; Wellington Onyenwe, MPH[14]; Ian Pray, PhD[19]; Sarah Reagan-Steiner, MD[20]; Sarah A. Reisinger, PhD[21]; Matthew D. Ritchey, DPT[13]; Mike Rollins, PhD[14]; Chariety Sapp, BS[14]; Stephen D. Soroka, MPH[22]; Noel Stanton, MS[19]; Daniel Y. Weng, PhD[3;] Ian Williams, PhD[15]; Jennifer Wiltz, MD[13]

Affiliations:

[1]California Department of Public Health, Richmond, CA; [2]Department of Internal Medicine, University of Iowa, Carver College of Medicine, IA; [3]Division of Environmental Health Science, College of Public Health, The Ohio State University, Columbus, OH; [4]Battelle Memorial Institute, Columbus, OH; [5]Bureau of Chronic Disease and Health Promotions, Arizona Department of Health Services; [6]Division of Human Development and Disability, National Center on Birth Defects & Developmental Disabilities, CDC; [7]Division of Reproductive Health, National Center for Chronic Disease Prevention & Health Promotion, CDC; [8]Health Systems and Research Branch, Division of Overdose Prevention and Control, CDC; [9]Agency for Toxic Substances and Disease Registry, Division of Community Health Investigations, CDC; [10]Department of Epidemiology and Environmental Health, University at Buffalo, Buffalo, NY; [11]Center for Surveillance, Epidemiology, and Laboratory Services, Deputy for Public Health Science and Surveillance, CDC; [12]Office of the Deputy Director for Non-Infectious Diseases, CDC; [13]Division for Heart Disease and Stroke Prevention, National Center for Chronic Disease Prevention and Health Promotion, CDC; [14]Division of Laboratory Sciences, National Center for Environmental Health, CDC; [15]Division of Foodborne, Waterborne & Environmental Diseases, National Center for Emerging & Zoonotic Infectious Diseases, CDC; [16]Minnesota Department of Health, St. Paul, MN; [17]Office on Smoking and Health, National Center for Chronic Disease Prevention and Health Promotion, CDC; [18]Division of Overdose Prevention, National Center for Injury Prevention and Control, CDC; [19]Wisconsin State Laboratory of Hygiene, University of Wisconsin-Madison, WI; [20]Division of High-Consequence Pathogens and Pathology, National Center for Emerging & Zoonotic Infectious Diseases, CDC; [21]Comprehensive Cancer Center, The Ohio State University and James Cancer Hospital, Columbus, OH; [22]National Center for Injury Prevention and Control, Deputy Director for Non-Infectious Disease, CDC

External Partners:

Arkansas Department of Health: Appathurai Balamurugan, Brandy Sutphin, James Allison, Gary Wheeler

Association of Public Health Laboratories: Scott Becker, Eric Blank, Julianne Nassif

California Department of Public Health: Kayla Thomas, Alon Volner, Julian Perez

Connecticut Department of Public Health: Diane Noel, Sydney Jones

Connecticut State Public Health Laboratory: Katherine A. Kelley

Hawaii Department of Health: All EVALI Staff

Illinois Department of Public Health Laboratory: Matt Charles, Carlos Morales, Mohammad S. Nasir

Indiana State Department of Health: Charles Clark, Brian Pope, Nicolas Epie, Eric Hawkins

Intermountain HealthCare: Sandeep Chowdhary, Dixie Harris

Los Angeles County Department of Public Health: Nicole Green

Maryland Department of Health: David Blythe, Kenneth Feder, Cliff Mitchell

Michigan Department of Health and Human Services: Matt Bashore, Rita Seith, Eden Wells

Minnesota Department of Health: Cory Cole, Paige D'Heilly, Kaila Hanson, Stacy Holzbauer, Mark Lunda, Ruth Lynfield, Jason Peterson, Stefan Saravia, Terra Wiens, Matt Wogen

Missouri State Public Health Laboratory: George Turabelidze, Valerie Howard

Monterey County Health Department: Kristy Michie

National Jewish Hospital: Russ Bowler

North Carolina State Laboratory of Public Health: Kate Koehler

Placer County Department of Health and Human Services: Lorene Vanzandt

Texas Department of State Health Services: Emily Hall, Varun Shetty

Utah Department of Health: Branden Ainsworth, Jordan Green, Rodney Goller, Jackie Patel

United States Food and Drug Administration: Duane Satzger, Rachael Schmidt

University of Iowa: Alejandro Comellas

University of Utah: Jeanette Brown, Sean Callahan

Wadsworth Center, New York State Department of Health: David Spink

West Virginia Department of Health and Human Resources: Shannon McBee, Lindsay Barr, Christy Reed

Wisconsin Department of Health Services: Meshel Lange, Jon Meiman
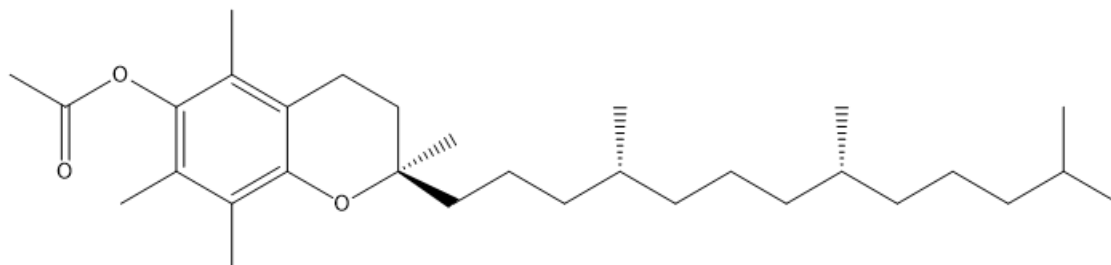
Figure S1. Chemical structure of Vitamin E acetate.

Table S1. Laboratory Methods for detecting toxicants of concern.

Potential Toxicant of Concern	Methods
Plant Oils	Plant oils were measured using mass spectrometry lipidomics analysis for long chain triglycerides (LCT)[1] with a limit of detection (LOD) of 2.0 ng/mL. Coconut oil is a plant oil, but it is identified by measuring medium chain triglycerides (see box below). Plant oils are mixtures of LCT. Specific fatty acyl (FA) patterns of LCT, based primarily on the relative abundance of triglycerides containing FA16:0, FA18:0, FA18:1, FA18:2, and FA20:4, were used to identify types of plant oils.[2] FA patterns for a variety of different plant oils are available.[2]
Medium Chain Triglyceride (MCT)-Oil and Coconut Oil	Triglyceride constituents of MCT-oil and coconut oil were measured using ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) with an LOD of 0.3 ng/mL. Observed fatty acyl patterns were compared to those of refined MCT-oil, liquid coconut, and coconut oil and based on the relative abundance of triglycerides containing FA6:0, FA8:0, FA10:0, FA12:0, and FA14:0. Given that hemolyzed red blood cells collected during bronchoalveolar lavage had the potential to introduce endogenous lipid into the sample, hemolysis was evaluated by hemoglobin measurement using a HemoCue® Plasma/Low Hb System (LOD of 20 mg/dL). Sixteen (11%) of BAL samples had traces of hemolyzed blood and their fatty acyl patterns aligned with those of serum/plasma.
Vitamin E Acetate (VEA)	VEA was measured using UHPLC-MS/MS with an LOD of 1.10 ng/mL.
Petroleum Distillates	Petroleum distillates were assessed based on the presence of *n*-hexane, methylcyclopentane, cyclohexane, *n*-heptane, and *n*-octane detected using headspace solid phase microextraction (HSPME) with gas chromatography (GC)-MS with respective LODs of 0.1, 0.02, 0.02, 0.1, and 0.1 ng/mL.[3]

Terpenes	Diluent terpenes (α-pinene, β-pinene, 3-carene, limonene, squalene, squalane) are terpenes that can be added to product fluid that enhance flavor or aroma. α-Pinene, β-pinene, 3-carene, and limonene were measured using HSPME with GC-MS/MS with respective LODs of 0.052, 0.052, 0.054, and 0.26 ng/mL. Squalene and squalene were measured by GC-MS with respective LODs of 0.225 and 0.225 µg/mL.
Cannabis-related Compounds	Cannabis-related chemicals were measured using UHPLC-MS/MS to assess the following set of cannabinoids: Δ9-THC, 11-nor-9-carboxy-THC (THC-COOH), 11-hydroxy-THC, cannabinol (CBN), cannabidiol (CBD) and 7-nor-7-carboxy-CBD. Respective LODs were 0.035, 0.019, 0.153, 0.030, 0.078, and 0.094 ng/mL.
Nicotine-related Compounds	Exposure to nicotine was assessed by measuring nicotine, cotinine and trans-3'-hydroxycotinine using UHPLC-MS/MS. Respective LODs were 0.050, 0.033, and 0.017 ng/mL.
Dipalmitoylphosphatidylcholine (DPPC)	DPPC was measured using UHPLC-MS/MS with a LOD of 1.34 ng/mL.

Supplemental References

1.   Cao Z, Schmitt TC, Varma V, Sloper D, Beger RD, Sun J. Evaluation of the Performance of Lipidyzer Platform and Its Application in the Lipidomics Analysis in Mouse Heart and Liver. Journal of proteome research 2019.
2.   Orsavova J, Misurcova L, Ambrozova JV, Vicha R, Mlcek J. Fatty Acids Composition of Vegetable Oils and Its Contribution to Dietary Energy Intake and Dependence of Cardiovascular Mortality on Dietary Intake of Fatty Acids. International journal of molecular sciences 2015;16:12871-90.
3.   Chambers DM, Blount BC, McElprang DO, Waterhouse MG, Morrow JC. Picogram measurement of volatile n-alkanes (n-hexane through n-dodecane) in blood using solid-phase microextraction to assess nonoccupational petroleum-based fuel exposure. Analytical chemistry 2008;80:4666-74.