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Pathology, toxicology, and latency of irritant gases known to cause bronchiolitis obliterans disease: Does diacetyl fit the pattern?

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ABSTRACT

Bronchiolitis obliterans (BO) is a rare disease involving concentric bronchiolar fibrosis that develops rapidly following inhalation of certain irritant gases at sufficiently high acute doses. While there are many potential causes of bronchiolar lesions involved in a variety of chronic lung diseases, failure to clearly define the clinical features and pathological characteristics can lead to ambiguous diagnoses. Irritant gases known to cause BO follow a similar pathologic process and time course of disease onset in humans. Studies of inhaled irritant gases known to cause BO (e.g., chlorine, hydrochloric acid, ammonia, nitrogen oxides, sulfur oxides, sulfur or nitrogen mustards, and phosgene) indicate that the time course between causal chemical exposures and development of clinically significant BO disease is typically limited to a few months. The mechanism of toxic action exerted by these irritant gases generally involves widespread and severe injury of the epithelial lining of the bronchioles that leads to acute respiratory symptoms which can include lung edema within days. Repeated exposures to inhaled irritant gases at concentrations insufficient to cause marked respiratory distress or edema may lead to adaptive responses that can reduce or prevent severe bronchiolar fibrotic changes. Risk of BO from irritant gases is driven substantially by toxicokinetics affecting concentrations occurring at the bronchiolar epithelium. Highly soluble irritant gases that cause BO like ammonia generally follow a threshold-dependent cytotoxic mechanism of action that at sufficiently high doses results in severe inflammation of the upper respiratory tract and the bronchiolar epithelium concurrently. This is followed by acute respiratory distress, pulmonary edema, and post inflammatory concentric fibrosis that become clinically obvious within a few months. In contrast, irritant gases with lower solubility like phosgene also follow a threshold-dependent mechanism of cytotoxicity action but can exhibit more insidious and isolated bronchiolar tissue damage with a similar latency to fibrosis. To date, animal and human studies on the highly soluble gas, diacetyl, have not identified a coherent pattern of pathology and latency that would be expected based on studies of other known causes of bronchiolitis obliterans disease.

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1. Clinical definitions of fixed obstructive lung disease and bronchiolitis obliterans disease

Obstructive lung disease is "characterized by an increase in resistance to airflow owing to partial or complete obstruction at any level from the trachea and larger bronchi to the terminal and respiratory bronchioles" [50]. In contrast, restrictive lung disease is caused by the "reduced expansion of lung parenchyma, with decreased total lung capacity" [50]. The major types of obstructive disorders are emphysema, chronic bronchitis, bronchiectasis, and asthma [50]. Several bronchiolitis diseases, including bronchiolitis obliterans (BO), are also considered obstructive lung diseases [50,73]. Each of these clinical disease entities, and a variety of more specific conditions, can occur in the same individual but each can differ greatly with respect to pathology, functional consequences, medical treatments, and known or suspected risk factors. In particular, there are several disease states and exposures that can lead to obstructive bronchiolar lesions and/or obstructive bronchiolitis obliterans (BO) disease, so attribution of BO disease to one particular exposure or risk factor may be scientifically tenuous. Additionally, diagnosis of BO disease is hampered by its similarity to other obstructive bronchiolitis conditions [73].

It should be noted that the terminology 'bronchiolitis obliterans syndrome' (BOS) was originally used to describe bronchiolar fibrosis occurring as a common element of tissue rejection and/or infectious insults in lung transplant patients who undergo pharmaceutical therapy to avoid tissue rejection [40]. In such cases, the newly implanted lungs may be diagnosed with BOS assumed to be caused by the complex interactions of the individual's immune system and hence lung biopsies are not considered necessary for diagnosis of BOS. Some authors of occupational investigations of lung disease have borrowed the BOS terminology to apply to diacetyl- or flavoring-related lung disease (e.g., [2,94]). However, such use of this terminology could be a misnomer in that, based on currently available information, no similar conditions (e.g., in terms of causal agents or risk factors) apply for BOS in lung transplant patients as distinguished from BO disease potentially related to occupational chemical exposures.

Fixed airway obstruction is a nonspecific and relatively common lung condition in people over the age of 50 with varied degrees of breathing difficulties that are not reversible with medications, e.g., bronchodilators for asthma or steroid treatment for acute lung inflammation [50]. Fixed airway obstruction, including BO disease, is a subset of conditions under the general category of chronic obstructive pulmonary disease or COPD [50]. Obstructive lung diseases and associated bronchiolar lesions may also occur in combination with or as a later consequence of restrictive lung changes, such as various diffuse interstitial lung diseases: pulmonary fibrosis, respiratory bronchiolitis, respiratory bronchiolitis-associated interstitial lung disease, hypersensitivity pneumonitis, collagen vascular diseases, and various types of pneumonia, including bronchiolitis obliterans organizing pneumonia (BOOP) and pneumoconioses (dust-related lung diseases) [50,52]. Bronchiolitis lesions can be found in association with these obstructive and restrictive clinical conditions, with overlapping imaging and histopathological features [17,73]. Although bronchiolar lesions represent permanent damage to the lung architecture, the vast number of small airways and excess oxygenation capacity of normal human lungs can provide a substantial buffer between early damage and later clinically significant disease with impairment [73]. As a result, many forms of bronchiolitis are described as indolent and unlikely to have fatal consequences; however, some acute bronchiolitis entities can result in more severe and deadly conditions, and chronic bronchiolitis can also evolve to BO disease [73].

Table 1
Generally accepted diagnostic criteria for bronchiolitis obliterans disease.

1. Pulmonary function tests show clinically important fixed obstructive deficits indicating small airways disease without appreciable impacts on total lung volume and gas exchange measurements.
2. The obstructive changes are resilient to treatment by corticosteroids or bronchodilators.
3. The patient's lungs show a mosaic pattern of attenuation on high resolution computed tomography (HRCT) scans indicating air trapping, especially during exhalation.
4. Lung biopsy shows definitive histopathology of widespread and severe concentric fibrosis of the bronchioles.

BO disease is a rare disease entity that specifically involves irreversible obstructive fibrosis of the small airways (bronchioles) [50,52,73]. BO disease is characterized by extensive fibrosis of the respiratory bronchioles that limits air exhalation or ventilation leading to reduced oxygenation especially with physical exertion [31,52,73]. The clinical course following inhalation of irritant gases known to cause BO disease initially involves acute respiratory distress associated with lung edema that occurs within days of sufficiently high exposure [50]. Onset of respiratory symptoms occurs without an appreciable delay, i.e., the latency between causal exposure and evidence of disease onset is relatively immediate [83]. However, BO disease may also demonstrate a longer latency due to other causes, such as when it develops as an exaggerated healing response following acute or chronic bronchiolitis [52,73] or during the late stages of hypersensitive pneumonitis [50,73]. Additionally, bronchiectasis, chronic emphysema, and chronic bronchitis can progress to BO disease [50,73]; thus determination of cause and effect can be very complex. In acute or chronically developed BO disease, clinically significant pulmonary function deficits and confirmed histology can be apparent. The diagnostic criteria for BO shown in Table 1 have been reviewed by several researchers with essentially concordant views [50,52,73,92,96,97].

The diagnostic criteria used for BO disease overlap with the manifestations of other lung disorders having variable etiologies. For example, mosaic lung attenuation has also been observed in asthma and emphysema [52,92]. There are also similarities with clinical presentation and pulmonary function testing between BO disease and other obstructive lung diseases [52]. In addition to a detailed medical and exposure history, a lung biopsy is often essential to distinguish BO disease from various other diseases potentially affecting the bronchioles [73].

Laohaburanakit and colleagues [52] noted that:

"In the case of BO that is not related to transplantation, the diagnosis is much more challenging and requires a high index of suspicion. The clinical presentation, as well as pulmonary function testing characteristics of BO, are non-specific and resemble other obstructive diseases such as asthma and COPD [chronic bronchitis and emphysema]. [···] Surgical lung biopsy, either by open lung biopsy or video-assisted thoracoscopic surgery (VATS) is usually required when BO occurs outside

of transplantation and the diagnosis cannot be made by transbronchial biopsy."

Chan and Allen [12] noted that:

"Lung biopsy is the only way to definitively diagnose BO, whether or not it is related to organ transplantation."

"Constrictive bronchiolitis" is recognized as a synonymous term for describing BO lesions. However, some investigators have broadened the definition of constrictive bronchiolitis to include indolent, subclinical, and/or relatively isolated bronchiolar lesions without fixed airway obstruction and with no appreciable impact on blood oxygenation or other objective clinical indicators of disease [32,48,47]. Furthermore, cases of possible subclinical constrictive bronchiolitis have not been confirmed by lung biopsy [33]. This broadened definition increases the difficulty in establishing cause and effect relationships because it encompasses other obstructive bronchiolitis entities, with varied risk factors, pathologies, and clinical severities.

For example, a clinical disease entity that often results in mixed obstructive-restrictive spirometry findings can be caused by long-term, heavy cigarette smoking: respiratory bronchiolitis-associated interstitial lung disease [50,84,96]. The less severe condition known as respiratory bronchiolitis or "smoker's bronchiolitis" is a common histological finding in the lungs of smokers and ex-smokers [50,84]. Respiratory bronchiolitis among smokers has subtle histological similarities to BO, with mild peribronchiolar fibrosis, but apparently develops more slowly and the bronchiolar changes typically don't play a critical role in the ultimate loss of normal lung function due to smoking and associated emphysema [50,73]. The interstitial lung disease component is characterized by the presence of mild fibrosis and pigmented intraluminal macrophages ("smoker's macrophages") within the first and second-order respiratory bronchioles [50]. Most instances of this disease are associated with mild clinical symptoms, mild to moderate obstructive-restrictive patterns on pulmonary function tests, and ground-glass attenuation on high resolution computed tomography lung scans (HRCT) [84]. This disease may occur in conjunction with emphysema in persons with over 30 pack-years of cigarette smoking, typically in their fourth or fifth decade of life [50].

Another disease that could be mistaken for BO disease if the broader definition of constrictive bronchiolitis was accepted is bronchiolitis obliterans organizing pneumonia or BOOP [96]. BOOP is also called cryptogenic organizing pneumomia and is considered primarily an interstitial lung disorder, in contrast to BO disease which is primarily a small airway disorder [17,28,50]. BOOP has been historically confused with BO due to its nomenclature; however, it is a clinical disease entity that is distinctly different from BO disease in terms of its pathology, diagnostic criteria, and associated risk factors [17,28,52,73,85,96]. BOOP may exhibit features of 'infiltrative bronchiolitis', describing serious acute infiltration of the bronchioles with immune cells typically in response to lung infections or an autoimmune disease [28,52]. BOOP can lead to fibrotic polyp lesions that may obliterate patchy areas of bronchioles, which only on biopsy can be distinguished from the concentric fibrotic lesions of the bronchioles seen in BO disease [52]. BOOP is most often a reversible acute condition causing restrictive (not fixed obstructive) lung changes and requiring antibiotics and corticosteroid treatment to resolve [50]. BOOP is not thought to lead to clinical BO disease; however, repeated and/or serious infections can result in BOOP leading to fibrotic bronchiolar lesions that upon biopsy may resemble patchy occurrence of BO lesions and/or bronchiectasis [17,28,85]. Indeed, BO and bronchiectasis lesions are commonly seen in late stages of many chronic lung diseases as explained by [73].

In sum, bronchiolitis diseases include a wide variety of pathologically unique entities, with overlapping histology and clinical symptoms. Accurate distinction between diseases is essential for predicting the clinical severity and progression of the obstructive symptoms. Furthermore, the etiologies of these various bronchiolar disorders vary significantly, from inhaled irritant gases or cigarette smoke to infectious diseases and autoimmune diseases [73]. As a result, failure to establish the correct diagnosis may lead to incorrect conclusions regarding cause and effect. Full consideration of the criteria and methods for diagnosis, the observed disease progression, and the patient's underlying conditions and alternative exposures is essential for proper diagnosis as well as for cause and effect determinations regarding BO disease.

2. Risk factors for restrictive and obstructive lung diseases and bronchiolitis obliterans disease

BO is a multi-factorial disease with critical host and non-occupational environmental determinants of risk [73]. Generally, a variety of known or suspected risk factors have been identified for restrictive and obstructive lung diseases that may include BO lesions, including cigarette smoke, fetal exposures, environmental exposures, pharmaceutical treatments, and infectious and autoimmune diseases. Since BO lesions are associated with a variety of chronic lung diseases, it is important to understand the differences in clinical features and risk factors between BO disease and other lung diseases.

The number of risk factors for obstructive lung disease can hamper epidemiology studies seeking to identify causative agents. Balmes [8] noted that exposures potentially causing chronic obstructive pulmonary disease (COPD) in the workplace have been difficult to define clearly in epidemiological studies for the following reasons:

"First, COPD is multi-factorial in cause with critical (and mostly unknown) host, as well as non-occupational environmental determinants, of risk. Second, unlike workers with pneumoconiosis, individuals with COPD caused by occupational exposures cannot be distinguished from those with disease resulting from other causes. Third, many workers with COPD have concurrent exposure to cigarette smoke (direct or second-hand) and workplace irritants. Fourth, exposed workers at baseline usually have better overall health and higher ventilatory function than the general population, the so-called healthy worker effect. Fifth, workforce studies are often limited to a survivor population because of the inability to assess or follow workers who leave their jobs, thereby underestimating the chronic effects of occupational exposures."

There is a broad range of agents that have been reported as known or suspected causes of either chronic bronchitis, bronchiolitis, BO disease, and/or similar small airways disease in humans, as illustrated for environmental exposures and disease states in Table 2 [3,5,8,10,12,52,56,68,73,79,85,87,97,106,110]. With respect to pharmaceutical or iatrogenic associations (see Table 3), most cases of BO disease are linked to heart/lung and bone marrow transplants and autoimmune connective tissue disease risk factors listed in Table 3, with the infectious agents more prominently affecting young children [12,17,79]. Additionally, many drugs used to treat BOOP and/or underlying diseases that may cause BOOP have been identified as possible risk factors (perhaps mistakenly) for BO disease in some individuals [5,10,29,106]. As noted earlier, repeated serious infections like BOOP may lead to BO lesions but not clinically defined BO disease [17,28].

2.1. Cigarette smoke

The predominant cause of chronic bronchitis, emphysema, respiratory bronchiolitis, and respiratory bronchiolitis-associated interstitial lung disease throughout the world is cigarette smok-

Table 2
Environmental exposures and disease states associated with bronchiolitis and/or bronchiolitis obliterans disease.

Irritant gases, fumes or dusts:
Ammonia, chlorine, hydrogen sulfide, mustard gas, smoke inhalation, sulfur dioxide, oxides of nitrogen (NO, NO_2, N_2O_4), phosgene, di-isocyanates, volatile flavoring agents, hot gases, fly ash, zinc chloride, metals (osmium, vanadium), metal oxide fumes (welding fumes), organic dusts (cotton, grain, wood), mineral dusts (coal, vitreous fibers, oil mist, Portland cement, silica, silicates), smoke (engine exhaust, tobacco smoke, fire smoke), overheated cooking oil fumes, spice dust.
Ingested toxins: *Sauropus androgynus*
Drug interactions: cocaine
Infectious and autoimmune diseases:
Chronic hypersensitivity pneumonitis
Childhood infections: (measles, respiratory syncytial virus, influenza, parainfluenza, adenovirus, mycoplasma, mycobacteria, pertussis)
Infections per se: (Herpes simplex virus, human immunodeficiency virus-1, *Cytomegalovirus*, *Rubeola*, *Parainfluenza* type 3, *Adenoviruses*, *Mycoplasma pneumoniae*, *Klebsiella*, spp., *Haemophilus influenzae*, *Bordatella pertussis*, *Mycobacterium chelonae*, *Nocardia asteroides*, *Cryptococcus neoformans*, *Pneumocystis carinii*)
Graft vs. host disease: (bone marrow, lung or heart-lung transplants)
Auto-immune connective tissue disorders: (rheumatoid arthritis, eosinophilic fascitis; polymyositis, cystic fibrosis with chronic infections, inflammatory bowel disease, Swyer-James syndrome, Sjogren's syndrome, Systemic lupus erythematosus)

Table 3
Pharmaceutical and iatrogenic factors associated with bronchiolitis and/or bronchiolitis obliterans disease.

Antimicrobials:
Minocycline, nitrofurantoin, cephalosporin, amphotericin-B, daptomycin, abacavir, tiopronin, lomustine, sulfasalazine, penicillamine
Anticancer agents:
Bleomycin, busulphan, doxorubicin, methotrexate, mitomycin-c, chlorambucil, cyclophosphamide, dihydroergocryptine, dihydroergotamine, hexamethonium, cytarabine ocfosfate, rituximab, oxaliplatin, aurothiopropanosulfonate, radiation therapy, Sauropus androgynus
Cardiovascular agents:
Amiodarone, acebutolol, pravastatin, simvastin, sotalol, ticlopidine, mecamylamine
Anti-inflammatory or immunosuppressive agents:
Gold, sulfasalazine, methotrexate, aurothiopropanosulfonate, infliximab mesalamine/mesalazine, bucillamine, D-penicillamine, azathioprine, 6-mercaptopurine, tacrolimus, sirolimus, everolimus
Anticonvulsants:
Carbamazepine, phenytoin
Miscellaneous drugs:
Interferons alpha, beta and gamma, hexamethonium, L-tryptophan, FK 506, barbiturates, nilutamide, tacrolimus, topolean, and free-base cocaine use, sulindac, ticlopidine, heroin, fluvastatin, venlafaxine, risedronate, lomustine

ing [84]. As noted above, mild fibrotic peribronchiolar changes are associated with respiratory bronchiolitis and respiratory bronchiolitis-associated interstitial lung disease [50]. Furthermore, chronic bronchitis and emphysema can progress to concurrently involve BO lesions but are not clinically defined as BO disease [50,52].

2.2. Fetal exposures

Maternal drug intake as well as premature birth (and associated hyaline membrane disease or bronchopulmonary dysplasia) may have substantial detrimental long-term effects on the lung [91,99]. Wang [98] noted that fetal exposure to nicotine from maternal smoking produces small airway changes in experimental animals and human fetuses that may affect bronchiolar function and disease risks in adulthood [13,64,100]. Thus, in some individuals a substantial portion of the cumulative lung damage that results in clinical obstructive lung disease during adulthood may date back to exposures or diseases affecting the fetus or newborn. Such risk factors may be unknown to the affected individual when onset of lung disease occurs decades later.

2.3. Possible role of chronic sinusitis in obstructive lung diseases

Irritant gases, particularly those with high water solubility, may be capable of causing chronic sinusitis that can lead to obstructive lung disease including small airways disease, bronchiolitis obliterans lesions, and bronchiectasis [51]. Chronic sinusitis involves the persistent occurrence of abnormal sinus drainage and associated swelling, pain, and associated problems for 12 weeks or more; it is usually associated with allergic rhinitis and changes in sinus and lung responses to allergens with increasing age [78]. In most cases, this condition is associated with chronic inflammation of nasal/sinus tissues related to abnormal increases in certain immune cells (eosinophils) that trigger swelling and edema in a manner that prevents normal drainage [11,14,78]. The chronic eosinophil-related inflammatory process is commonly related either to an allergic response (e.g., dust mite allergy of the respiratory tract), to abnormal immune responses (which may relate to nutritional, genetic, or age- and immune disease-related factors), or possibly to an anatomical abnormality of the nasal sinuses (e.g., congenital deviated nasal septum that can readily exacerbate sinus drainage problems).

Chronic sinusitis is extremely common and its prevalence increases with age, particularly starting around the fifth decade [54]; it is reported to affect more than 30 million people in the United States population [20], or about 1 in every 7 American adults [11]. Chronic sinusitis is highly prevalent in adults with severe asthma [105] and in persons who develop bronchiectasis and associated permanent obstructive lung changes [36].

Allergy testing is important because it is often the case that respiratory allergens can exacerbate chronic sinusitis and more severe cases (of allergic rhinitis) are linked to the occurrence of adult-onset asthma. For example, Magnan and colleagues [55] reported that 55% of asthmatics have allergic rhinitis, and that more severe respiratory allergies correlate with greater asthma severity and worse asthma control. Indeed, persistent allergic rhinitis is considered a strong risk factor for adult-onset asthma and it has been reported that up to 80% of patients with persistent allergic rhinitis have clinical asthma or a related condition called bronchial hyperreactivity [15,16,90]. Thus, adults who develop severe and persistent allergic rhinitis are at increased risk of developing chronic sinusitis and related asthma and/or bronchial hyperreactivity that may be a risk factor for permanent obstructive changes [55].

More severe cases of chronic sinusitis can increase the risk of permanent obstructive lung changes and specifically small airways disease with end stage changes that may also include bronchiectasis. Ragab and colleagues [80] reported that 60% of patients with chronic sinusitis had lower airway (bronchi, bronchioles, and alveoli) disease including 24% with clinical asthma and 36% with small airways disease (i.e., bronchiolar disease) as indicated by reduced spirometry measurements of forced expiratory flow at 25–75% (FEF_{25-75}). Lamblin and colleagues [51] studied 46 patients having persistent chronic sinusitis with nasal polyps and reported that non-reversible obstructive changes were observed on spirometry testing of most cases who were followed over a 4-year period. Greater obstructive changes were observed in 28 of the 46 patients who were considered to be 'non-responders' to aerosol steroid therapy used to treat the chronic sinusitis, and significant declines in spirometry parameters (FEV1, FEV/FVC ratio, and FEF_{25-75}) were observed for these patients regardless of whether or not they exhibited clinical asthma [51]. Guilemany and colleagues [36] reported that chronic sinusitis with nasal polyps was present in 25% of patients with clinical bronchiectasis, also an irreversible obstruc-

tive lung lesion. Thus, chronic sinusitis may be a risk factor for fixed obstructive lung disease.

In sum, there are many chronic lung diseases that in later stages can result in the occurrence of BO lesions that often have limited clinical significance and hence are not considered to represent BO disease. The wide variety of risk factors and commonality of COPD can make the determination of cause and effect relationships for obstruction from BO lesions difficult. Resolution of this problem is assisted by more specific definition of BO disease as widespread concentric bronchiolar fibrosis causing clinically significant respiratory impairment such as reductions in lung function and/or blood oxygenation due to unequivocal bronchiolar obstruction. This definition of BO disease generally avoids more equivocal or subclinical cases and is inferred in our use of this terminology going forward.

3. Time course and pathology associated with inhaled irritant gases known to cause bronchiolitis obliterans disease

Additional insights regarding the potential causes of BO disease can be gleaned from the magnitude of exposure and timing between the suspected causal exposure and the onset of airway injury responses. Schacter and colleagues [87] noted,

"Historically, airway injury has been one of the most common occupational hazards. The difficulty facing the physician who suspects an occupational airway disease is to sort out whether the illness is truly related to workplace exposure.

Bronchiolitis obliterans is a well-characterized inflammatory response of the terminal airways. Inflammation results from a usually massive exposure to irritant gases that penetrate to the lower airways (for example, nitrogen dioxide). Characteristically, after initial, relatively mild symptoms of mucous membrane irritation, pulmonary edema follows in an explosive manner. If the patient survives, bronchiolitis obliterans may develop. Frequently, a persistent, chronic obstructive lung disease ensues".

Schacter and colleagues [87] indicated that pulmonary edema and acute respiratory distress from "massive" exposures that may lead to BO disease are uniformly observed within days. This is in agreement with other reviewers on the sequelae of massive inhaled irritant exposures [30,31,52,56,73,103]. In such instances of acute respiratory distress from massive inhaled irritant exposure, do Pico [30] noted that the most common outcomes may include reactive airways dysfunction (an asthma-like condition) or complete recovery. BO disease is an uncommon clinical outcome. When it occurs due to acute inhalation exposures, BO disease develops quickly with a characteristic clinical course as described in Table 4 [30,44,52,81,103,104,107]. Thus, there is a general scientific consensus that traumatic acute exposures to certain inhaled irritant gases follow a predictable time course.

As explained earlier, some researchers have adopted a broader definition of 'constrictive bronchiolitis' and BO disease that includes more indolent forms without obstructive changes or clinically significant symptoms [47,58]. However, such a broad definition of BO disease provides little insight on causal elements and timing of disease onset, and such diagnoses are more likely to be confounded by idiopathic or secondary bronchiolitis conditions such as concurrent and/or repeated infections, underlying autoimmune or immune deficiency diseases, or other risk factors [73,98]. Similarly, the timing of onset for many forms of fixed airway obstruction is often dependent on the cumulative damage to the lungs that occurs as an end result of a variety of chronic exposures or disease states [98,101], e.g., chemical, physical, infectious, and other insults that may have occurred from fetal development or early childhood through the point where symptoms are sufficiently severe to lead to a doctor visit and diagnosis. Because the early signs of fixed airway obstruction—easy fatigue and shortness of breath on exertion—can be explained by common factors such as deconditioning, viral infections, and even depression, an early diagnosis is rare in the absence of spirometry screening or onset following a severe respiratory infection like pneumonia [52,96]. Moreover, the rare clinical entity that most researchers define as BO disease is characterized by confirmed fibrotic pathology of the bronchioles in the absence of other potentially causal disease processes [12,52,73].

The range of pathology findings that can be associated with varied doses of irritant gases reaching the bronchiolar epithelium is summarized in Table 5, ranging from normal healing or lung epithelium remodeling at low doses to severe bronchiolitis obliterans disease and possibly bronchiectasis at high doses. The potential for non-traumatic inhaled irritant gas exposures (i.e., those not associated with an acute respiratory distress syndrome within hours to days after the causal exposure) to cause BO disease years or decades later has also been discussed in recent literature pertaining to sulfur mustard gas exposures; associated clinical presentations range from unimpaired to severe impairment with respect to shortness of breath and lowered blood oxygenation [32,33,86,102]. The occurrence of BO lesions is not a definitive disease state unless there are clinically important fixed airway obstruction and/or impaired blood oxygenation related to bronchiolar fibrosis [12,52,73].

It is scientifically tenuous to attribute non-traumatic inhaled irritant gas exposures as a primary cause of BO disease. For example, the threshold-dependent mechanism of toxic action leading to BO disease from sulfur mustard gas is driven by cytotoxicity, DNA alkylation, and overwhelming of antioxidant defenses at doses sufficient to denude the respiratory epithelium, while lower doses do not cause bronchiolar fibrosis [4,9,46,57,60,83,95]. One must also consider that reported associations between non-traumatic sulfur mustard gas exposures and delayed BO disease have not been supported by biopsy confirmation [33], and hence other possible

Table 4
Time course of bronchiolitis obliterans disease from acute irritant gas exposures.

Within typically 1–3 days:
Lung edema/chemical pneumonia
Severe shortness of breath
Acute respiratory distress syndrome (ARDS)
Lymphocytic and neutrophilic infiltration
Within typically 3 weeks to 3 months (without timely steroid and antibiotic therapy):
Fixed obstructive lung changes from widespread concentric bronchiolar fibrosis
Superinfection and bronchiolitis obliterans obstructive pneumonia (BOOP)
Alveolar and upper airway lesions depending on agent and acute dosage

Table 5
Characteristic pathology findings of bronchiolitis obliterans disease from acute irritant gas exposures.

Low acute doses (not sufficient to denude bronchiolar epithelium):
With infrequent exposures, e.g., 2 week intervals—Normal healing and replacement of bronchiolar epithelium
With frequent exposures, e.g., daily—Remodeling of more sensitive cell types (e.g., clara cells) to less sensitive types
Threshold-dependent responses (doses sufficient to denude bronchiolar epithelium)
Severe bronchiolar inflammatory response
Cytotoxicity with severe basement membrane damage
Severe neutrophilic infiltration
DNA-alkylation and stunted healing and remodeling
Possible longer-term responses (without timely steroid and antibiotic therapy):
Widespread concentric bronchiolar fibrosis
Possible chronic bronchitis (purulent) from repeated infection
Possible bronchiectasis (in addition to concentric fibrosis) from repeated/chronic infection

causes of underlying fixed airway obstruction were typically not ruled out. Moreover, the influence of subsequent chronic infectious disease (e.g., chronic bronchitis and repeated pneumonia episodes) or other risk factors for fixed airway obstruction may provide more credible explanations for the development of BO disease years or decades later in persons not exhibiting an acute respiratory distress response soon after the causal exposure [93]. Similar considerations may apply to reports of delayed BO disease and other obstructive diseases in a group of soldiers exposed to a sulfur plant fire or burn pits during the recent war in Iraq [1,7,44,67].

Chlorine and phosgene are toxic gases that have been used as chemical weapons to induce rapid lung edema and act as 'choking agents;' they are also known inducers of BO [74]. Most survivors of high acute exposures to these choking agents have no long-term consequences with proper treatment, although exertional dyspnea and increased bronchial resistance may persist for several months to years [21,74,83]. Similar to mustard gas exposures, the mechanism of toxic action for phosgene-induced BO disease is driven by extensive cytotoxicity and denuding of the respiratory epithelium that only occurs with relatively high concentrations reaching the bronchioles [19,23,24,34,37,39,45,70,88]. The timing of potential development of BO disease following phosgene exposure is typically within months following traumatic exposures in humans [22,70] and is more rapid (i.e., typically within days) in certain laboratory test species [45,75,77,76]. Also, patients surviving the lung edema phase after traumatic phosgene exposure who do not receive preventive antibiotic therapy may develop "superinfective pneumonia" and repeated/chronic infections that can lead to bronchiectasis and/or BO disease [21]. It has also been noted that individuals with pre-existing lung damage may be at greater risk of developing BO disease years after traumatic phosgene exposures [22,70]. In such cases of pre-existing lung damage prior to a traumatic phosgene exposure, it may be difficult or impossible to determine which risk factors were most important in subsequent development of BO disease.

4. Adaptive response to irritants

Phosgene has been used as a research tool to understand the influence of adaptation on dose-response relationships for acute and chronic lung injury. With respect to fibrotic changes developing after traumatic phosgene exposures in animals, there are species-related differences in susceptibility (e.g., mice do not develop fibrotic bronchiolar changes while rats and dogs develop such changes within days to weeks) [24,39,45,77,75,76]. Rats appear to be about 10-fold more susceptible to phosgene-induced bronchiolar fibrosis compared to dogs or humans due to differences in lung anatomy and breathing characteristics [23,77]. In rats, it has been shown that pretreatment of animals with nontraumatic doses of phosgene will increase adaptation leading to greater phosgene tolerance, i.e., reduced occurrence/severity of lung injury compared to responses in newly exposed animals [34,39]. Hatch and colleagues [39] noted that such lung adaptation/tolerance reactions from phosgene pretreatment may be analagous to ultraviolet light exposures to human skin, where a series of smaller, nontraumatic exposures (e.g., inducing suntan but not sunburn) can be subsequently protective against sunburns that are strongly correlated with skin cancer risk. In the context of phosgene-induced bronchiolar fibrosis in rats, it is the number and timing of exposure events leading to significant cytotoxicity and denuding of the bronchiolar epithelium that equates with risk and severity of lung fibrosis; this is because regular low concentration exposures (e.g., daily or weekly events not capable of causing denuding of the bronchiolar epithelium) foster adaptation and greater tolerance to phosgene-induced lung injury [34,39].

5. Evidence on time course and pathology of diacetyl-induced lung damage

The likelihood that an inhaled irritant gas will reach the smaller airways at sufficiently high concentrations to cause clinically important tissue damage is essential with respect to the timing and localization of BO lesions or BO disease [73,74]. To evaluate this, it is necessary to understand how the physical/chemical properties of the suspected causal agent interact with the lungs. For example, Maier [56] noted,

"The effect of the substance inhaled depends on the physicochemical properties of the substance, with water-insoluble substances being more likely to reach the lung and cause acute lung injury. The pH, chemical reactivity, properties of the gas or aerosol, such as particle size and amount of substance inhaled or ingested, also determine toxicity."

Similarly, Schacter and colleagues [87] noted that,

"Not all gases that irritate mucous membranes are equally dangerous to the airways. For example, formaldehyde is potentially far more irritating to the mucous membranes of the eyes and nose than are sulfur dioxide or nitrogen dioxide, yet it has little or no airway effect at usual occupational levels, although sensitization may occur. Gases such as formaldehyde, which are highly soluble and are maximally absorbed in the upper airways, may not reach the lower airways in concentrations high enough to cause damage or irritation. The solubility of the gas and its ability to reach the lower airway is felt to be an important factor in promoting airway effects."

Thus, the more water soluble agents, such as acetaldehyde and ammonia gases, cause severe irritation of the eyes, nose and throat concurrent with effects occurring in the lower respiratory tract at the bronchioles.

Diacetyl vapors are highly water soluble and thus become absorbed primarily in the mucous of the nasal sinuses, trachea and bronchi [35]. Like ammonia, diacetyl is irritating to the mucous membranes of the eyes, nose, and throat at sufficiently high concentrations and is expected to cause irritation, acute inflammation, and associated avoidance responses in exposed individuals [103]. Thus, severe eye, nose, and throat irritation would occur at exposures to these agents that might cause clinically important tissue damage in the bronchiolar region. Highly water soluble irritants therefore usually have strong irritant properties that cause workers to avoid acute high exposures. In contrast, less water-soluble chemicals like phosgene and nitrogen dioxide have a greater potential for causing 'insidious' bronchiolar tissue damage, i.e., in the absence of frank signs of upper respiratory inflammation [21,103].

Diacetyl exhibits good warning properties due to its water solubility (up to 200 g/L; [61], pungent odor, and acute irritation effects on the mucous membranes of the eyes, nose and throat [38]. Upper respiratory irritants such as diacetyl and simple aldehydes (like formaldehyde, acetaldehyde, and acrolein) cause immediate inflammation of the mucous membranes of the eyes, nose, and throat at sufficient doses which leads to immediate avoidance responses in workers. Workers involved in jobs with intermittent exposures to irritant gases can learn how to avoid getting a stinging dose by using breath-holding, body positioning away from the emission path, and by using personal protective equipment or other techniques to prevent regular or substantial self-exposures.

Diacetyl is far more water soluble than phosgene [61]. Thus, while phosgene is capable of reaching the bronchiolar region at higher concentrations following inhalation [70] diacetyl becomes absorbed primarily in the nasal passages and bronchi of test animals. Diacetyl is also rapidly metabolized and eliminated from the body upon inhalation [35,65] and as a result does not participate in disease processes occurring months or years after diacetyl exposure has ceased. Gloede and colleagues [35] developed a pharmacokinetic model of rat and human respiratory uptake of diacetyl and reported that mouth-breathing humans under light exercise

might develop bronchiolar tissue concentrations 40-fold higher than those occurring in rats. However, as noted above for phosgene, the rat model may be at least 10-fold more sensitive to bronchiolar injury than dogs or humans due to differences in lung anatomy and breathing characteristics [23,76].

Consistent with phosgene research, diacetyl did not induce fibrotic bronchiolar changes in mice [62] but was capable of producing some degree of bronchial and/or bronchiolar inflammation in rats at sufficiently high inhalation doses, e.g., time-weighted average (TWA) diacetyl concentrations of 50 ppm or higher [71]. Since rats are considered to be at least 10-fold more sensitive than humans to bronchiolar inflammation from phosgene (due to lung anatomy and breathing physiology [21,76]), the TWA diacetyl concentration capable of causing similar bronchiolar responses in humans may be 500 ppm or higher. For context, surveys of food or flavoring workplaces that involve handling of concentrated diacetyl [108,109] typically involve TWA diacetyl concentrations in the low ppm range, e.g., between 0.1 and 3 ppm. Bronchiolar fibrotic lesions have also been reported to result from direct intratracheal instillation of diacetyl [72], although these investigators acknowledged that intratracheal instillation is not physiologically relevant human exposure pathway for diacetyl.

The timing of diacetyl-induced bronchiolar fibrosis following intratracheal instillation in rats [72] was comparable to that observed for phosgene (i.e., within days). Bronchiolar fibrotic lesions observed in some human cases associated with occupational diacetyl exposure have reportedly occurred within a span of a few months following start of exposure, although many suspected cases reportedly occurred many years after first occupational exposure and in workers with relatively low diacetyl exposures [48,47]. Assuming the rat studies on diacetyl intratracheal instillation [72] and of 2,3-pentanedione inhalation [63] are relevant to predicting fibrotic lung injury in humans, the mechanism of injury leading to bronchiolar fibrosis proposed by these researchers appears to be similar to that reported for phosgene. Specifically, development of bronchiolar fibrotic lesions induced by diacetyl in rats is thought to be driven by extensive cytotoxicity and denuding of the respiratory epithelium that is observed only at sufficiently high concentrations [48,47,63,72]. Unfortunately, available animal studies of diacetyl have not examined the potential for adaptation/tolerance to occur with regular exposures to lower concentrations, as has been observed for phosgene-induced lung fibrosis in rats [34,39].

In sum, there are many considerations with respect to the timing, pathology, and toxicology of diacetyl that do not fit the common pattern observed for better characterized agents like phosgene (see Table 6). Continuing research is therefore needed to determine whether or not diacetyl exposure is the true cause of the observed clusters of BO disease, or is simply a marker for other workplace exposure(s) that provide a more coherent scientific basis for establishing cause and effect.

Table 6
Characteristics of diacetyl chemistry and kinetics that don't fit with causation of bronchiolitis obliterans disease at plausible human exposure concentrations.

Relatively high water solubility (200 g/L):
Good irritant warning properties (eye, nose and throat inflammation, sensory irritant)
Implies good potential for learned avoidance for individuals with repeated exposures
Good tissue distribution for respiratory uptake
Low chance of insidious occurrence at the bronchioles
Rapid metabolism and excretion like a sugar/ketone body:
Accumulation at bronchiolar target tissue unlikely
Not a cumulative or insidious toxicant at the bronchioles
Threshold-type dose-response for epithelial tissue injury:
Poor dose-response correlation in epidemiology studies; marker vs. actual cause?
'Textbook' dose-response relationship for animal inhalation studies of acute irritants
Rats are 10-fold more sensitive than humans (bronchiolar inflammation at 50 + ppm TWA)
Based on rat lung inflammation response, human effective dose may start at 500 ppm TWA
Typical workplace exposure concentrations with handling of concentrated diacetyl: 0.1–3 ppm
Type of pathological responses should be comparable to other irritant gases:
Acute chemical burn to bronchioles seen in animals and humans with known causes
No bronchiolar fibrosis in diacetyl inhalation studies in animals, unclear in human studies
Latency to onset of pathological responses should be comparable to other irritant gases:
Rapid lung edema, ARDS, and bronchiolar fibrosis in animals and humans with known causes
Latency pattern inconsistent among human BO cases associated with diacetyl
No apparent mechanistic differences for cytotoxicity compared to other irritant gases:
Acute chemical burn to the bronchioles; no unique mechanistic factors to date
No known inter-individual susceptibility factors yet recognized:
No unique susceptibility factors identified to date

6. Diacetyl toixokinetics and mechanism

Kerger and colleagues [42] recently examined the influence of water solubility, diffusivity, metabolism, and other parameters on application of the Gloede and colleagues [35] respiratory tract dosimetry model to compare predictions of bronchiolar tissue concentrations of acetaldehyde, acrolein, and diacetyl. Table 7 provides a summary of the model parameters utilized, including kinetics parameters for acetaldehyde and acrolein reported by Asgharian and colleagues [49] and those for diacetyl reported by Gloede and colleagues. Substantial differences in water solubility, partitioning, and metabolism between the three compounds lead to distinctly different outcomes with respect to an example scenario used by Gloede and colleagues [35] involving prediction of bronchiolar tissue concentrations of diacetyl in mouth-breathing adults under light exercise conditions. As shown in Table 7, with an inhaled concentration of 1 ppm for each of these compounds in the model, acetaldehyde had predicted bronchiolar tissue concentrations nearly 4 orders of magnitude higher than those predicted for acrolein or diacetyl. This greater relative accumulation of acetaldehyde results from its much slower metabolism and greater resistance to mass transfer that causes greater deposition in the more distal respiratory epithelial tissues, despite its high water solubility [42]. If this finding is validated, then it is likely that acetaldehyde could be a considerably greater health concern than diacetyl or acrolein with respect to bronchiolar tissue cytotoxic injury and possible fibrotic disease affecting the small airways in humans. Since diacetyl may be a marker for, rather than the cause of, bronchiolitis obliterans disease in available epidemiological studies, it is important to consider such respiratory dosimetry information pertaining to diacetyl and other chemicals, like acetaldehyde and 2,3-pentanedione, which are common butter flavoring components used and produced in conjunction with diacetyl.

Just as tissue partitioning and metabolism can greatly affect the presumed target organ (bronchiolar) concentrations of irritant gases, recent findings by Scott and colleagues [89] point out the importance of liquid-to-air partitioning in exposure assessment of diacetyl in various flavoring mixtures. Specifically, Scott and colleagues [89] reported on headspace and small chamber studies showing that diacetyl added to soybean oil (as occurred in

Table 7
Respiratory tract dosimetry model parameters for acetaldehyde, acrolein, and diacetyl and model predictions for human bronchiolar concentrations.

Property	Acetaldehyde	Acrolein	Diacetyl
Diffusivity in air (cm^2/s)	0.128	0.105	0.091
Diffusivity in water (cm^2/s)	1.35E-05	1.22E-05	4.30E-06
Tissue:air partition coefficient	140	88	572
First order constant, K_p (1/s)	0.0357	0.05	0.005
Saturable pathway constant, K_m (ug/L)	1320	0.5	861
Saturable pathway rate constant, V_{max} ($ug/m^3/s$)	<1.0E-08	6.1E-07	0.964
Water solubility (g/L)	1000	212	200
Modeled concentration (ppm) in human bronchiolar epithelium with light exercise and mouth-only breathing at 1 ppm inhaled air concentration	74	0.0077	0.0077

Dosimetry model by Gloede and colleagues [35] for a mouth-breathing human under light exercise. Parameters for acrolein and acetaldehyde reported by Asgharian and colleagues [49] and for diacetyl as reported by Gloede and colleagues [35].

microwave popcorn factory worker exposures) led to unexpectedly high emissions of diacetyl when compared to aqueous or propylene glycol-water solutions used for adding diacetyl to dairy products and candies. They also determined that more dilute mixtures may emit disproportionately higher diacetyl concentrations (i.e., after normalizing to liquid diacetyl content) due to diminishing "like dissolves like" attractive forces at lower diacetyl concentrations (e.g., in solutions below 0.1%). Thus, diacetyl emission rates and exposures depend on the type of flavoring mixture and/or food to which it is added. Further research is needed to characterize the degree to which this may affect dosimetry in certain workplaces, e.g., those utilizing more hydrophobic and/or heated flavoring mixtures [89].

One pathway of possible genetic susceptibility for irritant gas lung toxicity was evaluated recently by Kerger and colleagues [43]. In this study, diacetyl and three other alpha-diketones were evaluated for their capability to cause activation or synergistic effects on the human Toll-like receptor 4 (TLR-4). Human embryonic kidney cells with either human TLR-4 or mouse TLR-4 were tested, but there was no evidence of receptor activation or synergistic effects with any of the tested diketones. TLR-4 receptor activity in humans is part of the "inflammasome" cascade [66] that can lead to excessive cellular immune responses and lung fibrosis in patients with certain autoimmune diseases like Crohn's disease, ulcerative colitis, and rheumatoid arthritis [5,18]. Possible connection to TLR-4 receptor activity was consistent with the observed time course studies of neutrophilic infiltration and bronchiolar fibrotic changes that followed diacetyl-induced denuding of the epithelium and disruption of the basement membrane in studies of intratracheal instillation [72]. Similar findings were reported for 2,3-pentanedione inhalation [63] in rats. Kerger and colleagues [43] showed that the human TLR-4 receptor does not appear to be involved in diacetyl or 2,3-pentanedione toxicity.

Other investigators have argued that alternative mechanisms might exist to explain potential inter-individual differences in human responses to diacetyl inhalation. For example, Hubbs and colleagues [41] argued that diacetyl may have biological interactions parallel to methyl glyoxal which is thought to play a key role in atherosclerosis in diabetes. Hubbs and colleagues [41] also reported that diacetyl may damage olfactory nerves in the nasal sinuses of mice. Larsen and colleagues [53] analyzed available rodent study data suggesting that diacetyl is a sensory irritant and that sustained human exposures over 20 ppm may cause sensory irritation. Morgan and colleagues [63] suggested that diacetyl may cause injury to the airway epithelium by alteration of cellular proteins containing arginine, and considered that hapten formation might act as a susceptibility factor for causing BO disease in humans [59]. Also, Egilman and colleagues [26,27] considered the possibility that diacetyl and toluene di-isocyanate may share the same chemical reaction mechanism based on analysis of molecular orbital energies, and considered the hapten hypothesis as an explanation for respiratory bronchiolitis interstitial lung disease possibly related to diacetyl in chronic cigarette smokers. Dworak and colleagues [25] conducted molecular orbital energies analysis of diacetyl and toluene di-isocyanate and concluded that diacetyl is unlikely to have significant respiratory sensitization potential. Dermal sensitization from diacetyl has been demonstrated in mice [6,82], but there is no known connection between dermal sensitization and pulmonary disease. In sum, these studies to date have failed to identify strong, specific and coherent alternative mechanistic considerations that advance the understanding of diacetyl-related respiratory effects at relevant exposure concentrations in humans. Regardless of the mechanism leading to cytotoxicity, the bronchiolar fibrosis is triggered by sufficient acute insult causing denuding of the epithelium down to the basement membrane followed by neutrophil infiltration and fibrotic tissue replacement of the bronchiolar epithelium [63].

In summary, diacetyl has been demonstrated to have certain physical/chemical and kinetics-related characteristics (see Table 6) that help to explain why the animal inhalation toxicology studies to date have failed to demonstrate bronchiolar fibrosis at plausible human exposure concentrations. Each of these factors, in addition to workplace exposure assessment differences, must be taken into careful consideration when assessing whether or not a suspected chemical like diacetyl is a cause of bronchiolitis obliterans disease in humans.

Competing interests

BDK and MJF are employed by Exponent, Inc., a United States firm that provides scientific research and consulting services under contracts to industry and government clients. MJF is also employed by the Center for Occupational and Environmental Health at the University of California, Irvine, an academic medical institution where he conducts research and performs medical examinations and medical intern training. BDK and MJF have provided research and consulting services to clients through their employers including medical monitoring of patients with workplace flavoring and other chemical exposures (MJF) and in relation to health claims in litigation (MJF and BDK). Funding of this manuscript was solely through the authors' employers and no outside party provided input regarding the study design, methods, interpretations, or conclusions of this work. Opinions expressed in this manuscript are solely those of the authors and not necessarily of their employers.

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Author contributions

BDK and MJF each independently researched the topics described in this manuscript and BDK wrote the first draft. MJF reviewed the draft manuscript and provided substantial further

input and modifications. MJF and BDK finalized the manuscript and both stand behind the technical content, interpretations, and conclusions expressed.

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