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Diacetyl - A Critical Safety Review of the Science Defining the Environmental Inhalation Hazards Association with Chronic Lung Disease

Franklin L. Mink [a++*]

[a] *USEPA AD, United States.*

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Review Article

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ABSTRACT

In the last two decades scrutiny of several retrospective occupational studies on performed by NIOSH in the early 2000's on ambient indoor air exposures to the flavoring chemical diacetyl and more complex butter flavoring formulations has led to a reported association between diacetyl and severe irreversible lung disease, primarily bronchiolitis obliterans. A group of laboratory rodent studies, performed primarily by associated researchers, followed in the next two decades with the intent to determine a plausible physiological mechanism for bronchiole scarring applicable to the human respiratory tract. Recently, a renewed interest in diacetyl as a flavoring constituent of vaping liquids and marijuana and inhalation exposures has emerged. This paper reviews the universe of published literature to date in relation to whether diacetyl or butter flavors containing diacetyl causes occupational or environmental lung disease, more specifically BO (i.e., general causation) and whether specific levels of inhaled diacetyl or butter flavors containing diacetyl are associated with chronic lung disease.

[++] *Retired;*
**Corresponding author: E-mail: glufmink2@yahoo.com;*

The review included numerous journal articles, government reports, etc. Based upon the evidence, while the literature reflects a statistical association between both diacetyl and butter flavoring diacetyl mixtures and lung disease, there is sufficient evidence to conclude that general causation between exposure to diacetyl concentrations measured in the ambient air environments studied to date and chronic lung disease does not exist.

Keywords: Inhalation; ambient air; environment; bronchiolitis obliterans; butanedione; epidemiology; toxicology.

1. INTRODUCTION

Diacetyl (2,3-butanedione or $CH_3COCOCH_3$, CAS # 431-03-8) is a chemical compound with a yellow-greenish color produced by the metabolism of sugars via pyruvate as a natural process of fermentation. It has been chemically synthesized for over 80 years being widely used in flavorings in over 6000 food/beverage products and is a common contaminant in ambient air. The principal types of flavorings that use diacetyl are those meant to mimic dairy flavors, particularly “butter” and “cheese” flavorings. Diacetyl is sometimes an ingredient in caramel, butterscotch, and coffee flavors. The diacetyl-containing flavors may be found in popcorn, wine, snack foods, baked goods, cooking oils and candies. Diacetyl was evaluated and affirmed as a generally recognized as safe (GRAS) direct food additive in the 1980s by the FDA (21CFR184.1278). The FDA confirmed that diacetyl was safe for ingestion in beverages and food under the intended conditions of use [1,2]. During cooking food and beverages such as butter, oil and wine emit airborne diacetyl (see Table 1).

Although a statistical association between both diacetyl and diacetyl containing butter flavoring mixtures and lung disease has been reported the underlying data in all the occupational studies from 2000-2008 were flawed and unreliable (NIOSH Method 2557 in complex mixtures}. Actual diacetyl concentrations of up to 300 ppm in directly inhaled tobacco smoke or ambient air concentrations have not been demonstrated to cause occupational or consumer chronic lung disease, more specifically BO (i.e General causation) - There is insufficient evidence to conclude general causation between exposure to diacetyl or butter flavoring and lung disease as diacetyl is also common and naturally occurring air pollutant from the fermentation process of the human skin (perspiration/sweat) especially around the head, neck and feet producing an unpleasant body odor in small concentrations of approx.. 5– 6000ppb [3,4,108,109) The Flavor and Extract Manufacturers Association (FEMA) reported that the Research Institute for Fragrance Materials [18] found that the acute dermal exposures (LD50) in Rabbits was greater than 5000 mg / kg – body weight diacetyl In another investigation by Hubbs et al, [19], the finding was, “Diacetyl Inhalation caused Epithelial Necrosis and Suppurative to Fibrosuppurative Inflammation in the Nose, Larynx, Trachea, and Bronchi. Bronchi exhibited affects at diacetyl concentrations of 294.6 ppm and greater, and the trachea and larynx exhibited affects at diacetyl concentrations of 224ppm are greater”. NIOSH Researchers later (110) examined the hypothesis that vapors of butter flavoring used in the manufacturing of selected microwave popcorn and other foods can produce air way injury in rats but complex butter flavoring containing 285-371 ppm diacetyl caused necro suppurative rhinitis affecting all four levels of the nose. Controls and lower airways were unaffected. Concentrations of butter flavorings that can occur in the manufacturing of foods are associated with epithelial injury in the nasal passages and larger pulmonary air ways of rats only at levels exceeding those accurately measured at any manufacturing plant. The researchers concluded that acute exposure to diacetyl alone was sufficient to cause Upper Respiratory Tract Epithelial Necrosis in Rats at concentration of 198.4ppm or higher but not in lower airways. Van Rooy et al. [20,21] in relation to the prevalence of lower respiratory symptoms concluded a relationship could not established between lung function abnormalities and exposure to diacetyl [20,21]. NIOSH researchers [22] assessed diacetyl emissions and air borne dust levels from butter flavoring used by several microwave popcorn manufacturing companies and reported that diacetyl was one of the most abundant

Table 1. Diacetyl emissions from food

Food Item	Range of naturally occurring diacetyl content inhaled from foods (ppb except where noted)	Reference
Butter	480 to 4000	Bakirci et al. [5], Chrysan [6]
Cottage cheese	20 to 4000	Antinone et al. [7]
Chedar cheese	230 to 760	Drake et al. [8]
Coffee	2660 to 2780	Daglia et al. [9]
Goat milk Jack cheese	5970 to 13680	Attaie [10]
Margarine	480 to 27000	Rincon-Delgadillo et al. [11]
Heated margarine	7000 to 180000	Schneider [12]
Wine	200 to 7000	Davis et al. [13]
Yogurt	200 to 16700	Baranowska [14], Cheng [15]
Heated butter	7000 to 160000	Cruz et al. [16], Güler et al. [17], Schneider [12]

compounds found in all the flavorings but lowering all plant emissions by implementing engineering controls to minimize exposures would reduce the respiratory problems of the workers in the absence of toxicity data In the 2014 Surgeons General's Report on Smoking BO was not mentioned as a smoking-related disorder despite the levels of diacetyl in tobacco smoke with inhalation of natural or flavored tobacco is approximately 100 times the highest levels of diacetyl inaccurately measured in the Jasper GML Plant air samples.

2. LITERATURE REVIEW

2.1 Acute Animal Toxicity

The early scientific literature regarding diacetyl was scant and dominated by acute toxicity studies, primarily intended to determine LD50 and LC50 values, the lethal oral dose (LO) or lethal inhaled concentration (LC) at which 50 percent of the test population dies. For example, Jenner et al. [23] reported an oral LD50 for diacetyl administered by gavage of 1,580 mg/kg-bodyweight for rats and 990 mg/kg for guinea pigs [24]. It should be noted that the point of these studies is to see how much of a substance is lethal in 50% of the test animals and is solely used as a measure of overt, gross toxicity (111) demonstrated the preferential partitioning of diacetyl between fat and water.

The Flavor and Extract Manufacturers Association (FEMA) reported that the Research Institute for Fragrance Materials [18] found that the acute dermal LD50 in rabbits was greater than 5,000 mg/kg-bodyweight diacetyl, based on the death of l of the 8 test animals at that dose [25]. Irritation was also evaluated as part of the study. The researchers applied a 24-hour occluded patch to intact and abraded skin and read reactions according to Draize. Diacetyl was observed to produce moderate (7 of 10 rabbits) to marked (3 of 10 rabbits) erythema and moderate (9 of 10 rabbits) to marked (8 of 10 rabbits) edema [25].

The RIFM researchers again evaluated dermal irritation in 1988 in a 4-hour semi-occluded patch test conducted on 4 female New Zealand white rabbits. A 0.5 mL aliquot of neat diacetyl was placed on a 2.5-cm2 piece of surgical lint, which was subsequently applied to intact skin on the left flank of each of the test animals. Reactions were read at 1, 24, 48, 72 and 168 hours following patch removal. The researchers considered a substance an irritant if the average score for erythema or edema equaled or was greater than 2. The erythema score was 0.6 and the average edema score was 0. Based on their observations, the researchers classified diacetyl as a non-irritant [25]. FEMA/RIFM
researchers conducted an eye irritation test for diacetyl in three rabbits. A 0.1 ml aliquot of pure diacetyl was distilled into the left eye of each rabbit as a one-time treatment, with the right eye serving as a control. Reactions were scored according to Draize at 1, 4, 24, 48, 72 and 96 hours and again at 7, 14 and 21 days. Diacetyl was observed to produce severe irritation and thus was classified as a class III corrosive material (i.e., inducing a corrosive effect with no recovery within 21 days) [25]. The rabbit is a sensitive species model for eye irritation.

Researchers at BASF [26], unpublished report) exposed Wistar rats to diacetyl vapor for 4 hours at 3 concentration levels: 2.25 mg/L, 5.2 mg/L,

and 23.9 mg/L. No mortality occurred at the low concentration group, whereas all animals died following exposure to the mid and high exposure concentrations. Therefore, the LC50 for both male and female animals was estimated to be: 2.25 < LC50 < 5.2 mg/L (the low exposure group of 2.25 mg/L is equivalent to approximately 2250 mg/m3 or 633 ppm average). The mid and high concentration groups showed hyperemia of the lungs and edema of the lungs and bronchi. The mid concentration group also exhibited moderate emphysema and focal hyperemia of the lungs.

Colley and others [27] established two LD50s (oral and intraperitoneal) for acute diacetyl exposure in rats. A 20% solution in water was administered by oral intubation or intraperitoneal injection. The oral LD50 was between 3,000 mg/kg of body weight (females) and 3,400 mg/kg (males), whereas the intraperitoneal LD50 was between 400 mg/kg (males) and 640 mg/kg (females).

Morgan et al. [28] subjected mice to oropharyngeal aspiration (inserting a tube down the nasal cavities and injecting liquid directly into the lungs) of 100, 200 and 400 mg/kg-bodyweight diacetyl. The researchers observed foci of fibrosis without inflammation at the junction of the terminal bronchiole and alveolar duct in all mice at the highest dose that survived to day 4 after treatment and in one of five mice treated at the intermediate dose.

Morgan and collaborators [28] also treated three groups of mice with a one-time dose of 100, 200, and 400 mg/kg-bodyweight, respectively, administered by oropharyngeal aspiration. The purpose was to circumvent the upper airways effects observed due to the highly developed (large anatomically compared to humans) nasopharynx of the mouse. The mice in the 400 mg/kg dose group exhibited foci of fibrohistiocytic proliferation with little or no inflammation at the junction of the terminal bronchiole and alveolar duct. They were sacrificed and processed 4 days following the one-time dose. The authors noted that the one-time dose resulted in fibrohistiocytic lesions in the distal airways of mice. They stated that fibrohistiocytic foci were present at the junction of the terminal bronchiole's alveolar ducts with minimal inflammation in a small number of treated animals. They stated that since these lesions developed in only 4 days after a single dose, they extrapolated these results to suggest that diacetyl may be highly fibrogenic in the small airways. Although these early lesions did not obstruct the small airways. They determined that the results are suggestive of early stages of BO.

Hubbs et al. [19] investigated the toxicity of inhaled diacetyl in rats at concentrations of up to 365 ppm (time weighted average or TWA), either as six-hour continuous exposures or as four brief, intense exposures over six hours. A separate group inhaled a single pulse of -1800 ppm diacetyl (92.9 ppm six-hour average). Necropsy of the test rats occurred 18 to 20 hours after exposure. Diacetyl inhalation caused epithelial necrosis and suppurative to fibrosuppurative inflammation in the nose, larynx, trachea, and bronchi. Bronchi exhibited effects at diacetyl concentrations of 294.6 ppm or greater, and the trachea and larynx exhibited effects at diacetyl concentrations of 224 ppm or greater.

2.2 Animal Toxicity Studies (Subchronic and Chronic)

Colley and others [27] performed a subchronic study (90 days) using administered doses, via oral intubation, for 5 groups: 0 mg/kg body weight (control), 10, 30, 90, and 540 mg/kg/day. The animals were sacrificed and autopsied after 90 days. Chemical analyses were performed on the subjects' blood and urine. Extensive pathological evaluations were also performed. The NOEL was identified as 90 mg/kg body weight/day for 20% diacetyl solutions. The authors estimated the average daily human intake of diacetyl from various dietary sources and concluded that the NOEL observed in their studies was 500 times higher than the expected human dietary intake circa 1969.

In response to popcorn workers reported as developing lung disease following potential exposures to flavoring vapors, NIOSH researchers [29] examined the hypothesis that vapors of butter flavoring used in the manufacture of selected microwave popcorn and other foods can produce airway injury in rats. The researchers exposed Sprague-Dawley rats for a total of 6 hours to vapors liberated from heated butter flavoring. The rats were necropsied one day following exposure. The vapors were analyzed by GC-MS and found to be a complex mixture of volatile organic compounds (VOCs), with the major peaks consisting of diacetyl, acetic acid, acetoin, butyric acid, acetoin dimers, 2-nonanone, and alkyl acetones. Diacetyl was used as a marker of exposure concentration based on its peak size using NIOSH Analytical

Method 2557 [30]. The researchers concluded that the butter flavoring vapors containing 285-371 ppm diacetyl caused multi-focal necrotizing bronchitis, mostly present in the main stem bronchus with the alveoli unaffected. They concluded that butter flavoring containing 285-371 ppm diacetyl caused necrosuppurative rhinitis, affecting all four levels of the nose. Controls were unaffected. The researchers then concluded that concentrations of butter flavoring that can occur in the manufacture of foods are associated with epithelial injury in the nasal passages and larger pulmonary airways of rats. These studies were consistent with the 1993 BASF study of Wistar rats, which showed no effects on the lung below 65 ppm, but above approx. 100 ppm hyperemia of the lungs with edema and bronchi with moderate emphysema were observed (but no nasal effects were observed). It should also be noted that the murine (rat) anatomy is significantly different than the human nasal bronchial architecture, making comparisons to human effects uncertain, as noted by several researchers since 2002; and these levels are significantly above maximum occupational exposure levels measured by later validated analytical methods.

Hubbs et al. [31] studied acute inhalation exposure to diacetyl alone rather than to heated butter flavoring as investigated in their earlier work [29]. The researchers concluded that acute exposure to diacetyl alone was sufficient to cause upper respiratory tract epithelial[1] necrosis in rats at concentrations of 198.4 ppm or higher.

Kreiss [32] reported that the National Institute for Environmental Health Sciences (NIEHS) conducted subchronic studies to evaluate diacetyl exposures in mice, and additional studies in rats were being planned. Based on evidence of rodent nasal toxicity, the NIEHS researchers [28] limited inhalation exposures to 1 hour per day, 5 days per week. The researchers reported chronic bronchitis, laryngitis, and rhinitis after 2 and 4-week exposures.

Morgan et al. [33] followed up on their 2006 studies with a study of the respiratory toxicity of diacetyl in a mice using several exposure profiles they suggested as relevant to workplace conditions at microwave popcorn packaging plants. Male C57B I /6 mice were exposed to inhaled diacetyl across several concentrations and duration profiles, or by direct oropharyngeal aspiration (see acute toxicity section). Effects were evaluated by histopathology and bronchoalveolar lavage (BAL) analyses. Subacute exposure to 200 to 400 ppm diacetyl for up to 5 days caused deaths, necrotizing rhinitis, necrotizing laryngitis and bronchitis. Reducing the exposure to 1 hr./day at concentrations of 100, 200, and 400 ppm for 4 weeks resulted in less nasal and laryngeal toxicity but led to peribronchiolar lymphocytic inflammation. A similar pattern was observed with intermittent extreme high dose exposures at 1,200 ppm (15 minutes, 2 times/day, 4 weeks). Subchronic exposures to 100 ppm (6 hr./day, 12 weeks) caused moderate nasal injury, and peribronchial lymphocytic inflammation accompanied by epithelial atrophy, denudation, and regeneration.

Hubbs et al. [19] reported that both pulsed and continuous exposure patterns caused epithelial injury. The authors contended that these findings were consistent with the conclusion that inhaled diacetyl is a respiratory hazard. NIOSH's complicated extrapolations of data in marginal animal models violate the basic principles of toxicology/pathology.

Clark and Winter summarized the later animal studies on diacetyl as follows: Animal inhalation toxicity studies have also been used to investigate the hypothesis that butter flavoring vapors (BFV) are causal to respiratory injury if inhaled at levels relevant to potential exposures in the workplace. Hubbs and others [29] exposed male Sprague-Dawley rats (n = 19 control, 18 BFV-exposed) to BFV for 6 h. Individual rats (200 to 250 g) were caged within 20 × 16 × 14 inch (0.07 m3) whole-body exposure chambers. For comparison, this is similar to a 100 kg (220 lb) human being confined in a small 35 m3 (12.5 × 12.5 × 8 ft) office for 6 h. Low exposure was defined as 203 ppm, middle exposure was defined as 285 ppm, high constant exposure was defined as 352 ppm, and high-pulsed-exposure was defined as 371 ppm, ranging from 72 to 940

[1] *Fedan et al. (2006) investigated the effects of diacetyl on the epithelium of guinea pig isolated airway tissue preparations and the effects of diacetyl in vitro on reactivity to bronchoactive agents. They inferred from their findings that diacetyl exposure compromised epithelial barrier function, leading to hyperreactivity to mucosally applied methacholine. In light of this, the authors extrapolated the in vitro animal tissue results to suggest that intact respiratory epithelium in humans appears to serve as the initial target for the toxic effects of diacetyl in the airways. The suggestion that these tissue preparations subjected to diacetyl vapors (by direct saturation as tissue preparations not "inhaled" substances) may mirror human inhalation exposures.*

ppm. The levels selected for the study were unrealistic extremes. Even the “low” exposure level was about 10000 times higher than the recommended short-term exposure limit (TLV-STEL: 0.02 ppm) for humans [34]. Under these conditions, the rats experienced inflammatory responses, including necrosis of nasal and airway epithelium; 2 died after exposure. The authors concluded that the NOAEL for a 6-h exposure to butter flavoring lies below the levels used in the experiment. However, it could be argued that the “low” level was thousands of times higher than where they should have started for a more realistic experiment.

2.3 Industrial Hygiene and Human Epidemiological Studies

Van Rooy et al. [20] from the Netherlands Expertise Centre for Occupational Respiratory Disorders (NECORD) and the Institute for Risk Assessment Sciences (IRAS) at Utrecht University conducted a retrospective study in a Dutch diacetyl production plant among workers who were potentially exposed to diacetyl in the period from l960-2003. The cohort was defined as all workers ever involved in diacetyl production with no limit to a minimal employment time. A total of 206 present and former workers were identified by the company as potential participants, 10 of whom had died (no investigation was done in relation to their deaths). All 196 remaining workers, present and former, were invited to participate, 174 (89%) of whom responded and participated. The participants all filled out a questionnaire and underwent spirometry evaluation. Workers with observed airway obstruction were referred for clinical evaluation. The researchers accepted a diagnosis of BO in cases with a fixed airway obstruction and a HRCT scan exhibiting air trapping with hypo attenuation in segmental or lobular areas and mosaic pattern of perfusion, yet no biopsied confirmations were reported.

Three cases of BO were reported, all process operators. The prevalence of respiratory symptoms among workers potentially exposed to diacetyl was compared to a sample of the Dutch general population. The worker population experienced significantly more trouble with breathing, daily cough, and asthma. Interestingly, however, there were no apparent differences in measured lung function. A relationship could not be established between lung function abnormalities and exposure to diacetyl [20,21].

Akpinar-Elci et al. [35] -- NIOSH researchers performed a study as a follow-up to the 2002 work by Kreiss et al. [36] regarding the lung disease cases observed at the Jasper Mo. Gilster Mary Lee (GML) Plant. The researchers reviewed the clinical details of 9 (versus 8 reported) former workers diagnosed with BO. Eight HRCT scans showed marked bronchial wall thickening and mosaic attenuation with air trapping. Lung biopsies were performed on 3 of the cases. According to Akpinar-Elci, two of three biopsies showed results indicative of constrictive bronchiolitis. Five cases were reported to be on lung transplant lists at the time of the article's publication but no follow-up on transplant status was reported.

The authors reported that some workers appeared to have shown slow improvement of their fixed obstruction after cessation of work at the plant. The authors conceded that pathological uncertainty remained, but they asserted that the following clinical features were still most consistent with BOS: fixed obstruction, largely normal chest radiographs, and bronchiectasis and air trapping on HRCT. The researchers observed that the pathophysiology is elusive and recommended that it may be best approached through studying animal models and clinical investigation of any future cases with bronchoalveolar lavage studies.

Akpinar-Elci et al. [37] also performed another study one year following the initial cross¬ sectional study at the Jasper GML Plant reported by Kreiss et al. [36]. The study's aim was to determine whether exhaled nitric oxide (FENo) levels were associated with exposure levels, respiratory symptoms, or airways obstruction in the workers at the Jasper GML Plant. A questionnaire, spirometry, and FENo measurements were completed by 135 workers at the plant. The FENo levels for the high exposure group (n = 107) were compared to levels for the low exposure group (n = 28) and low healthy external controls (n = 31). Comparisons were made, controlling for smoking status since smoking results in lower measured FENo levels. The researchers concluded that FENo levels were not a useful diagnostic for lung injury in flavoring-exposed workers. Interestingly, they observed lower levels of FENo in the high exposure group than in the low exposure and control groups. Low FENo levels generally represent pathologic changes in airways in lung diseases other than asthma that are not

accompanied by eosinophilic or lymphocytic inflammation.

NIOSH researchers [38] performed another study using the Jasper GML Plant data focusing on the characterization of potential respiratory exposures at the plant. The authors reported that workers in the production area were exposed to particulates and a range of over l00 different organic vapors. Particulates consisted mainly of salt and oil/grease particles. Analytical results identified the same volatile organic compounds referenced in prior articles. The authors pointed to diacetyl as a contributor to observed lung disease, also noting that contributions of other specific compound(s) associated with obstructive lung disease in these workers was still unresolved.

NIOSH researchers [39] analyzed data from medical and environmental surveys at six microwave popcorn plants, including the Jasper GML Plant studied by Kreiss et al. [36]. The study had 3 main goals: (1) explain how the concentration of diacetyl relates to the specific work type performed by employees at the study plants; (2) relate the level and duration of exposure to butter flavoring chemicals, and other factors such as smoking history, to respiratory tract symptoms, airway dysfunction, and lung biopsy findings of bronchiolitis; and (3) describe practical measures that may decrease workplace exposure to butter flavoring chemicals.

The study concluded that respiratory symptoms and airways obstruction prevalence were higher in oil and flavorings mixers, especially those with longer work histories (> 12 months), and in packaging-area workers near non-isolated tanks of oil and flavorings. Workers were affected at 5 plants, one with mixing area exposures to diacetyl as low as 0.02 ppm. On this basis, without explanation of the reported oil and particulate exposures, the authors recommended that microwave popcorn processing plants try to maintain average diacetyl concentrations below this level, integrate engineering controls and provide personal protective equipment (PPE) to workers. They also speculated that peak exposures may be hazardous even when ventilation maintains low average exposures.

NIOSH researchers [22] assessed diacetyl emissions and airborne dust levels from butter flavorings used by several microwave popcorn manufacturing companies. They heated bulk samples of 40 different butter flavorings (liquids, pastes, and powders) to approximately 50°C and used gas chromatography to measure the relative abundance of VOCs emitted. Over 150 VOCs were identified. The researchers reported that diacetyl was one of the most abundant compounds found in all the flavorings. Other compounds commonly detected were acetoin and 2-nonanone, butyric acid and acetic acid. The researchers also conducted air sampling for diacetyl and for total and respirable dust during the mixing of powder, liquid, or paste flavorings with heated soybean oil at a microwave popcorn plant. To further examine potential exposure to the powders, they simulated manual handling of powdered buttered flavoring and measured the emissions. Powder flavorings were found to yield much lower diacetyl emissions than pastes or liquids. The authors encouraged companies that use butter flavorings to consider using flavorings with lower diacetyl emissions, and to implement engineering controls to minimize exposures. They also encouraged that employees be required to wear PPE until engineering controls were implemented.

Researchers from the United States Environmental Protection Agency ([40], USEPA), conducted a study in response to the earlier retrospective NIOSH work at the Jasper GML Plant. The authors indicated that NIOSH scientists reported that workers in the Quality Control (QC) areas of several popcorn plants where workers pop bags of popcorn for QC purposes, have shown an increased risk of lung disease [39]. In response to this concern, the USEPA study characterized chemicals released into a chamber in the process of cooking microwave popcorn. The researchers studied 17 types of microwave popcorn from 8 different brands. The study proceeded in two phases: Phase l investigated chemicals emitted during popping and opening, while Phase 2 investigated chemicals emitted at discrete intervals from 0-40 minutes post-pop bag opening. The researchers performed the analysis using a microwave oven enclosed in a chamber with ports for air sampling of particulate matter (PM) and VOCs.

The compounds observed during popping and opening included components such as diacetyl, butyric acid, acetoin, propylene glycol, 2-nonanone, and triacetoin and bag components such as p-xylene and perfluorinated alcohol. The researchers observed that the greatest chemical quantity is emitted when the bag is immediately opened post-popping. In fact, more than 80% of the total chemical emissions occur at this time.

The researchers observed that except for the particulate matter, the emissions of chemicals from a single bag of microwave popcorn ultimately appear to be low, often within an order of magnitude of the detection limit of the GC/MS.

Dr. James Lockey and colleagues presented a paper at the Central States Occupational Medicine Association meeting in Indianapolis, Indiana on September 20, 2008 regarding "Airway Obstruction Associated with Diacetyl Exposure at Microwave Popcorn Production Facilities." Lockey et al. indicated that in 2002 an index case was identified at a microwave popcorn plant owned by ConAgra, referred to in the article as "Plant L" The authors said this finding, as well as identification by NIOSH of two additional mixers with airways obstruction, led to a company-wide medical surveillance study beginning in 2005 for four plants across the country. The presentation represented the results of this medical surveillance study across the four plant sites from 2005 through 2006 for then current employees. Employee exposures to diacetyl were assessed through personal, breathing zone air sampling starting at Plant L in 2003 and for the other plants in February 2005. Initial spirometry testing was conducted at 4 facilities every 4 months (February 2005 - January 2006). Ongoing surveillance was performed once per year. Spirometry testing at the plants met American Thoracic Society (ATS) criteria and used Hankinson et al. [41] normal predicted values. A medical questionnaire was completed by study participants that covered upper and lower respiratory symptoms and occupational history. Participating employees with obstructive pulmonary disease were identified for additional pulmonary follow-up. Lockey et al. linked medical surveillance data with comprehensive ongoing industrial hygiene monitoring and job group classifications for study participants. Exposure parameters evaluated for study participants included the following: total production years; diacetyl production years; dichotomous cumulative mean diacetyl exposure; mixing room exposures vs. all other exposures, subdivided into Pre-PAPR and Post-PAPR. The designation of Pre- and Post-PAPR relates to the fact that in April of 2003 all ConAgra plants required employees working in the mixing room to wear a powered air purifying respirator (PAPR-112) calculated and adjusted for age (< 40, > 40) and smoking (current/ former/ never smokers) the ratio of the number of observed cases of an obstructive PFT pattern in the cohort to the number of expected cases based upon urban NHANES III data (https://wwwn.cdc.gov/nchs/nhanes/nhanes3/default.aspx) and reported by Kreiss et al. [36]. Cumulative diacetyl exposure for individual workers was dichotomized into higher (> 0.8 ppm-yrs.) and lower (< 0.8 ppm-yrs.) exposure groups.

Lockey et al. (2008) concluded that this study of workers involved with manufacturing microwave popcorn at four US production facilities demonstrated no statistically significant impact of diacetyl exposure on FEV l predicted values in non-mixing room employees. Also, there was no increase in an obstructive PFT pattern by age or smoking category in the overall study cohort. In contrast, they observed a significant impact on percent predicted FEV l in males who worked as mixers within the slurry room where butter flavorings containing diacetyl were added to heated soybean oil. These changes occurred at lower mean diacetyl concentration levels than that found in the previously reported study of the Jasper GML Plant first studied by Kreiss et al. [36]. The estimated change in percent predicted FEV 1 was in those employees with >0.8 ppm cumulative diacetyl exposure as compared to those with < 8 ppm cumulative exposure. Despite several years of medical monitoring, no cases of BO were found as a result of the Lockey et al. study. Dr. Lockey, in subsequent litigation deposition testimony, indicated that mixers with obstructive lung disease required clinical follow-up to determine the nature and cause of their condition (Deposition of James Lockey, M.D. in the matter of George T. Aldrich II et al., flavoring plant employees v. International Flavors & Fragrances, Inc. et al., Defendants, Case No. A070045 I, November 5, 2008).

Martyny et al. [42], investigators at the National Jewish Medical and Research Center, Denver, CO, consulted with several individual flavor manufacturing companies to determine exposures and lung health risk among flavor manufacturing employees. Beginning in 2003, the researchers at National Jewish worked with 16 flavor companies to (a) characterize exposures to chemicals of concern (including diacetyl, acetoin, acetaldehyde, benzaldehyde, and acetic acid); (b) recommend appropriate engineering controls to reduce exposures; and (c) provide respiratory protection programs in smaller flavor companies if deemed appropriate. Companies that participated in the study were in eastern, Midwestern. and Western states, with the largest number in California, where state

OSHA efforts have encouraged flavor companies to seek industrial hygiene assistance. The authors began their industrial hygiene evaluation at each facility by obtaining information about facility layout, products utilized, health and safety plans, and employee numbers. They then arranged with each company to conduct air sampling on product mixtures that used either high percentages (> l %) of diacetyl or required heating diacetyl. This approach was designed to obtain worst-case diacetyl exposures during the sampling period. The mean diacetyl concentration for all the process samples was 1.80 ppm and the median was 0.10 ppm. The median diacetyl levels by company ranged from a low of <0.01 ppm to a high of 1.50 ppm. The authors stated that powder compounding operations had the highest maximum exposure levels in contrast to other studies, with a high of 52.5 ppm measured during that process. Mean levels also appeared to be higher during powder operations than during liquid compounding operations, suggesting an extended period of exposure for these operations. The authors stated their data suggest that diacetyl levels in small to medium sized flavor manufacturing facilities widely variable, ranging from the limit of detection (<0.01 ppm) to as high as 60 ppm, with a mean of 1.80 ppm and a median of 0.10 ppm. The authors concluded that results of exposure monitoring in 16 flavor companies show that peak airborne levels of diacetyl may be like reported levels in other diacetyl-using industries where cases of fixed obstructive lung disease were reported. They claimed their findings suggest that powder operations result in the highest exposures in flavor production areas. These results were inconsistent with earlier NIOSH findings [22] that found higher diacetyl emissions from liquid and paste flavor formulations. Compared with the microwave popcorn industry, there is wide variability in frequency and duration of use of diacetyl among flavor companies, with likely lower 8-hr TWA exposures overall. They suggested that further investigation of peak, 8-hr, and cumulative exposures and their association with risk for obstructive lung disease is necessary.

Van Rooy et al. [43] presented their findings from an epidemiological survey of a historic cohort of workers from a diacetyl production plant. The purpose of the study was to investigate and reconstruct exposures, respiratory symptoms, lung function, and exposure-response relationships by modeling available exposure data. The authors indicated this investigation was conducted in the same plant that was addressed in the earlier referenced Van Rooy [20,21] works, and they noted the epidemiologic description of the cohort in this study as being complementary to the case reports of BOS. The authors performed a cross sectional study in 2005 in a cohort of former workers in a chemical plant producing diacetyl in the Netherlands in the period 1960-2003. The plant was closed in 2003. The Human Resources department for the plant identified 206 workers who potentially had been exposed to diacetyl in that period of whom 10 had died. The authors located the remaining 196 workers and obtained written, informed consent from 175 (89%) for study participation. The authors had study participants complete a questionnaire to provide details on work history and work-related symptoms, applicable to both the diacetyl plant (job title, duration and number of days/week) and other plants at the production site (type of plant, type of occupational exposure, exposure duration and frequency - number of days/week). In addition, workers were asked if they experienced exposure incidents, e.g., during maintenance and/or process disturbances. Experienced technicians obtained spirometric lung function variables in all participants according to European Respiratory Society standards. The authors used data from the Dutch part of the ECRHS, a general population sample, as a reference for comparison. The authors stated that compared to the Dutch ECRHS population, diacetyl plant workers reported significantly more continuous trouble with breathing, daily cough, self-reported asthma attacks, physician diagnosed asthma attacks, and having had an asthma attack in the last year. Compared to a minimally exposed internal reference group, operators (including three BOS cases) and quality control lab workers reported significantly more trouble with breathing, and operators reported also significantly more shortness of breath in the last year. The authors stated that despite these results regarding symptoms, there was no clear association between FEV1 (% predicted) and exposure to diacetyl. On the contrary, multiple linear regression analysis of pulmonary function variables on exposure in process operators showed a significant increase in actual FEV1 of 28 ml per year working in the diacetyl plant before 1995 (95% CI,3 -53) and a significant increase in FEV l of 2 ml per cumulative weighted number of years in the diacetyl plant (95% CI, 0 -4). The authors described several findings that they characterized as robust as well as illustrative of the occupational hazard of

diacetyl: (1) the highest exposed group, the process operators, had significantly more respiratory symptoms than other occupational groups, and the plant population as a whole had excess symptoms compared to the general population; (2) a previously published spirometry analysis internal to the cohort showed that process operators had a job title-related decrement in FEV I (-292 ml); and (3) all reported BOS cases occurred in process operators.

In 2008–2009, NIOSH studied high-quality lung function tests in 106 employees in a flavoring manufacturing facility in Indiana. Mean tenure was 16.2 (range: 0.2–36) years. The diacetyl concentrations were obtained by established methods (OSHA PV2118 and 1012), which showed a geometric mean 8-hour time-weighted concentration in the range < 0.001–1.9 ppm and with a maximum of 2.9 ppm. There were also measured exposures to other compounds. Abnormal spirometry was observed among 32 % of employees. Restrictive patterns were observed among 30 subjects (28%), which was mild, moderate, moderately severe and severe among 22, 6, 1 and 1 employees, respectively. Any obstructive pattern was observed in 3 %; 2 employees had mild and 1 had moderate obstruction. A severe mixed pattern (obstructive + restrictive) was observed in 1 employee. The prevalence of restriction was 3.8 times that in the US population when compared to urban areas via NHANES data. Among 70 employees with high quality spirometric test, 13 (19%) had an excessive FEV1 decline and abnormalities were apparently progressive. The annual decline in FEV1 was 2.8 times greater and the abnormal decline occurred about 7 times more frequently in the highly exposed compared with employees in other areas. They suggested the observed restrictive reactions may be due to other compounds than diacetyl, which had been reported to appear mainly associated with obstructive abnormalities [44].

The raw data used by Kreiss et al. [44] were re-analyzed by J. Pierce et al., [45] and also found an increased prevalence of restriction (29%). The prevalence risk [odds ratio (OR): 3.3] was significantly increased compared to the US population. However, the prevalence of restriction was not related to exposure levels. Thus, 27% of the highly exposed and 33% of the low-exposed had airway restriction. Neither tenure in a work area with high potential for exposure (OR: 0.97) nor tenure in liquid compounding (OR: 0.99) was associated with increased prevalence. Similarly, no increase was observed in those who had ever worked in a job with high potential for exposure (OR: 0.84) or ever worked in a liquid compounding area (OR: 0.72). Additionally, the decrease in lung function (FEV1 and FVC) was analyzed by general estimating equation (GEE) models. The GEE models did not indicate an association between lung function decline and tenure at the facility [p = 0.46 (FEV1) and 0.90 (FVC)], tenure in work areas with high potential for exposure (p = 0.13 and 0.40, respectively) or tenure in liquid compounding (p = 0.56 and 0.997, respectively). The authors questioned the NHANES U.S. urban population comparison as being an appropriate control group for the worker cohort, which consisted mainly of males aged 30–60 years, of which about a third were obese, and many were rural residents with significant farming exposure. Therefore, workers in the group with low potential for exposure were considered more appropriate for comparison, which gave the conclusion that “many years of exposures to flavoring chemicals in this workplace, including diacetyl, were not found to produce an increased risk of abnormal (mainly restrictive) spirometric findings” [46].

NIOSH researchers (113) examined the methodologies to determine excessive short-term decline in forced expiratory volume in one second (FEV1) in diacetyl-exposed workers. They evaluated five methods of determining excessive longitudinal FEV1 decline in diacetyl-exposed workers and workers from a comparative cohort: American Thoracic Society (ATS), ACOEM, an 8% limit, and a relative and absolute longitudinal limit on the basis of spirometry data variability. The researchers evaluated relative risk and the incidence of excess decline. Incidence of excessive FEV1 decline was 1% in the comparative cohort using ATS and ACOEM criteria, 4.1% using relative limit of longitudinal decline, 4.4% with absolute longitudinal limit of decline, and 5.6% by using the 8% limit. Relative risk of abnormal FEV1 decline in diacetyl-exposed workers was elevated in all evaluated methods. (Kreis et al., 2010).

In 2012, Egilman et al. reported respiratory exposures to diacetyl and diacetyl-containing flavorings used in butter-flavored microwave popcorn (BFMP) based primarily on the Jasper studies were stated as “causes lung disease, including bronchiolitis obliterans (BO), in flavorings and popcorn manufacturing workers. However, there are no published reports of lung

disease among BFMP consumers." The authors presented a case series of three BFMP consumers reportedly having biopsy-confirmed BO. They reviewed data relating to consumer exposures, estimated case exposures, and compared them to diacetyl-containing flavoring-exposed manufacturing workers with lung disease. These consumer cases' exposure levels are comparable to those that were reported as causing disease in workers. They did not identify any other exposures or diseases known or suspected to cause BO in these cases. They stated, "Some manufacturers have substituted diacetyl with other α-diketones that are likely to pose a similar risk. Simple consumer practices such as cooling the popcorn bag would eliminate the risk of severe lung disease." These case studies did not provide any information as to heated oil being a known causative agent of BO or present the relevant medical histories of the consumers.

Clark and Winter also reported a comprehensive review of occupational and human studies for diacetyl safety. They stated that the concern about the potential of diacetyl to cause pulmonary disease associated with MW popcorn began in May of 2000 with an unusual cluster of fixed airway obstruction cases reported in workers in a MW popcorn plant in Missouri [36,19]. Missouri Department of Health reported eight workers who had formerly worked in the plant as having BO [47,36]. Investigators from NIOSH [36] conducted medical examinations and environmental surveys of workers and concluded that the estimated cumulative diacetyl exposure correlated with lung disease in a factory. The lowest levels of diacetyl based on area sampling in the plain popcorn packaging line, bag printing areas, warehouse, offices, or outside was 0.04 parts per million parts air by volume (ppm); the highest level of diacetyl measured was in the mixing room, where area sampling was 32.23 ppm. Exposure levels to particulates and organic vapors in the same plant as investigated by Kreiss and others [36] were measured by Kullman and others [38]. Over 100 different volatile organic compounds were isolated in the environment. Observed diacetyl concentrations in the plant were reported as ranging from below detection limits to a high of 98 ppm, with a mean concentration of 8.10 ppm (SD 18.5 ppm). Geometric mean corrects for skew, so it is a more realistic representation of data; the geometric mean diacetyl exposure level was much lower, 0.71 ppm (SD 14.4 ppm) [38]. Since the initial investigation in Missouri, NIOSH has conducted at least 16 additional industrial hygiene and medical Health Hazard Evaluations related to diacetyl in plants focused on food production, food preparation, and flavoring manufacturing (NIOSH 2015).

Investigators of an Ohio MW popcorn plant (115) measured the mean TWA (time weighted average) diacetyl air concentration in the slurry room to be 1.14 ppm. The mean TWA diacetyl air concentration in the packaging area was 0.02 ppm (Kanwal and Kullman 2004). Later, researchers (14) returned to the first implicated plant in Missouri for follow-up lung function tests and air sampling. The diacetyl air concentration in the mixing area (highest worker exposure area of all locations), which ranged from 2.3 to 98 ppm in 2000, had a geometric mean of 26 ppm. The diacetyl air concentration in the quality control area (which is most similar to, though not the same as a consumer setting) ranged from 0.33 to 0.89 ppm (geometric mean 0.49 ppm). In subsequent visits (2001, 2002, and 2003), levels of diacetyl in all locations had dropped significantly, with measures not exceeding 5.9 ppm in any location; most measurements were many orders of magnitude smaller.

3. DISCUSSION

3.1 Key Occupational Studies and Interventions

Select members of the scientific and medical community have concluded that exposures to diacetyl alone or to complex butter flavors containing diacetyl can cause fixed, obstructive lung disease, specifically BO. However, in this discussion we consider whether reliable, recognized diagnostic criteria, reproducible toxicological assessments, industrial hygiene studies, epidemiological reviews and/or analytical data support this conclusion.[2]

[2] *"A causal relationship is based on 'evidence [that] is sufficient to conclude that there is a causal relationship between relevant pollutant exposures and the health outcome. That is, a positive association has been observed between the pollutant and the outcome in studies in which chance, bias, and confounding could be ruled out with reasonable confidence. Evidence includes, for example, controlled human exposure studies; or observational studies that cannot be explain by plausible alternatives or are supported by other lines of evidence (e.g. animal studies or mechanism of action information). Evidence includes replicated and consistent high-quality studies by multiple investigators.' ISA Table 1–2, at 1–11." 75 Fed. Reg. 35,525 (June 22, 2010).*

The initial association between diacetyl and/or diacetyl-containing butter flavors and BO originated from an investigation of the Jasper GML Plant in 2000 that was prompted by the report of litigation expert, Dr. Alan Parmet, that 8 or 9 former workers of that Plant had bronchiolitis obliterans. As a result of Dr. Parmet's report, NIOSH evaluated the Jasper GML Plant and 117 of the 123 then current workers of the Plant (the exclusion of 6 healthy workers is statistically significant, adding to potential study bias). However, that investigation contained a multiplicity of documented errors (analytical, clinical, epidemiological, toxicological, pathological and regulatory), which resulted in a series of decisions (see NIOSH, Final Draft Diacetyl, 2016) that led to the postulation that diacetyl was the most probable causative agent responsible for BO at the Jasper GML Plant. Some of the initial errors in this foundational NIOSH investigations influenced subsequent related published studies, possibly leading to confirmation bias.

One major issue was that the NIOSH air sampling Method 2557 that was eventually discovered to have serious reliability issues was used as the primary analytical method for all of NIOSH's Human Health Evaluations (HHEs) performed before late 2008 for measuring airborne diacetyl (including the NIOSH HHEs for all of the 5 sentinel flavoring plants, HETA #2001-0474-2943, July 2004). Until 2007, this was the air sampling and analytical method for diacetyl used in the field, but it is no longer recommended for use [48]. In 2007, NIOSH first recognized, through field and chamber investigations, that Method 2557 was adversely affected by humidity, resulting in an unreliable quantitation of true diacetyl concentrations. To evaluate the sampling and analytical methods for diacetyl, a field comparison study between new and existing sampling collection methods was conducted [48]. Parallel field samples were collected and analyzed according to the following methods: NIOSH Method 2557, OSHA Method PV2118 (another method under development by OSHA for specific concentration ranges of diacetyl), and a modified version of the OSHA method in flavoring manufacturing facilities. The results of this field work confirmed the tendency of the NIOSH method to incorrectly estimate the true concentration of diacetyl. However, no mathematical correlation was found in this data set that would produce an adjustment factor to allow for correction of results.

As a result, NIOSH researchers collaborated with scientists at the OSHA Salt Lake Technical Center laboratory to study the effects of humidity on measured diacetyl air concentrations using NIOSH 2557, to compare sampling methods, analytical methods, and field procedures. These collaborating agencies reported that their evaluation was conducted as part of an investigation of occupational exposures to diacetyl and other food flavorings at two flavoring manufacturing facilities. The primary objective of these surveys was to characterize potential occupational exposures within the flavoring industry by identifying common work tasks, plant processes, and procedures. The researchers compared the same three sampling and analytical methods for diacetyl in workplace air. The authors reported that NIOSH Method 2557 incorrectly estimates true diacetyl concentrations as relative humidity increases. Both temperature and relative humidity were significantly correlated with a greater difference in air concentration results (i.e., greater unreliable quantitation by the NIOSH method) produced by the different diacetyl air sampling methods. These data suggest that both the temperature and relative humidity at the time of collection, and not moisture content alone, are important to this phenomenon. The authors recommended that NIOSH method 2557 not be used to determine the concentration of airborne diacetyl. Until a new method was developed, NIOSH investigators were mandated to discontinue use of NIOSH Method 2557 (as it could not be fully validated due to an unacceptably high error rate of over 25% despite over 8 years of continuous development and testing) and use the newly modified OSHA method and collect measurements for both temperature and relative humidity during investigations or research studies [48].

In a 2014 summary of the history of NIOSH's methods for air sampling and determination of airborne concentrations of diacetyl, the EU Scientific Committee on Occupational Exposure Limits [49] observed that under NIOSH Method 2557 the limit of detection (LOD) may be as low as 2.3 ppb (8.3 µg/m3). In summarizing the comparison of sampling methods by Ashley et al. [48] SCOEL noted of NIOSH 2557 that laboratory recovery at 0.5 ppm diacetyl ranged from less than 10% to 100%, depending on ambient humidity. Highest recoveries were observed for high diacetyl concentrations and were less influenced by ambient humidity. While Ashley et al. [48] developed a mathematical correction

procedure for estimated diacetyl concentrations based on laboratory studies, SCOEL noted that this procedure does not account for the potential influence on recovery posed by other airborne chemicals [50], nor does it reliably correct concentrations below the detection limit [49]. SCOEL [49] also noted that the NIOSH method does not capture particulates or particulate bound diacetyl.

The SCOEL evaluation of NIOSH Method 2557 was undertaken to determine exposure-response relationships from epidemiological studies where it had been used for exposure assessment to obtain information on the order of magnitude of the bias of the NIOSH Method 2557 [49]. SCOEL compared analytical results from both NIOSH and OSHA methods available from the field studies in two facilities that mixed and formulated food flavorings reported by Ashley et al. [48]. The OSHA methods (48,116) had higher detection rates than the NIOSH method, the latter method showing many results below the detection limit where the unbiased methods detected diacetyl in the air [49]. Thus, SCOEL concluded from evaluation of these data that it was not possible to establish a fixed correction factor for adjustment of results obtained with the NIOSH method. SCOEL further concluded it was possible to obtain an idea about the magnitude of the bias of the NIOSH method by comparing results on airborne diacetyl levels that were detectable by both methods, although such a comparison is only appropriate for high concentration levels where the least bias is expected [50]. The comparison shows that a result obtained by means of the NIOSH method may incorrectly estimate the true concentration by a factor of 1–13 [49]. In the high-concentration range, correction by a factor of 3 may both under- and overestimate the bias of results obtained with the NIOSH method, depending on the many parameters previously discussed. For example, SCOEL stated that Kanwal et al. [51] and NIOSH (117) re-evaluated original data from the Jasper GML Plant investigation [36] where 40% of the samples had diacetyl levels below the LOD. The NIOSH Method 2557 reportedly results in unreliable quantitation by a factor of 20 for concentrations below the LOD with a range from 4.2 to 295 [49].

A recent expert report by USEPA in 2016 for Ethylene Oxide Inhalation Cancer Risk Evaluation echoed a long-known fact in assessing occupational risk, “Nonetheless, errors in retrospective exposure assignments are inevitable, and exposure estimation is a primary source of uncertainty in the unit risk estimates. Thus, the unit risk estimates based on the NIOSH study could over-predict or under-predict the true risks to an unknown extent.” This was referring to high quality retrospective epidemiological studies. NIOSH Method 2557 has been shown to be inaccurate and unreliable for measuring diacetyl even when appropriate QA/QC procedures are carefully followed. Method 2557 is also unreliable for identification of diacetyl without additional analytical support.

Diacetyl analysis using Method 2557 does not accurately measure the effects of relative humidity, temperature, light stability, filter material, sampling tube material, sampling flow rate, and hold times on the air concentrations (OSHA Methods Manual, Method 1012/1013). Without accurate measurement of these effects, the method can overestimate or underestimate air concentrations of diacetyl, yielding irreproducible and unreliable results. Therefore, conclusions drawn from air sampling results in key NIOSH studies such as the sentinel Jasper GML Plant at the outset of investigations into diacetyl and diacetyl containing butter flavoring should be viewed with this in mind. The investigation of the Jasper GML Plant failed to positively identify and quantify the complex mixture of volatile compounds in the air, it failed to exclude respirable aerosols and dust particles (primarily comprised of oil and salt) as a cause of reported lung conditions, and it failed to consider the study population’s pre-employment and outside employment exposures. NIOSH’s subsequent investigations between 2000-2008 (and reanalysis of the same data after 2008) using the same methods perpetuated these same problematic issues. In addition, the conclusions of other researchers who relied on the original Jasper GML Plant investigation should be viewed in light of these issues.

Investigators have concluded that corrective actions (including better ventilation, PPE, and isolation of tanks containing flavorings) reduce exposure and protect employees (117, 118). Collaborating investigators who conducted work on behalf of NIOSH at MW popcorn production plants in Illinois (120), Ohio (Missouri [39], and Montana (120,121)) helped produce the recommendation by the ACGIH for strict limitations to occupational diacetyl exposure. A TLV-TWA of 0.01 ppm (0.04 mg/m3) and a TLV-STEL of 0.02 ppm (0.07 mg/m3) have been proposed by the ACGIH for occupational

exposure to diacetyl (122). However, ever since the release of the first NIOSH report, various investigators have questioned whether diacetyl was the causal agent leading to lung disease in MW popcorn plant employees. Taubert and others [52] questioned whether diacetyl alone can be singled out as a causative agent in lung ailments in a Letter to the Editor regarding Kreiss and others [36]. Since then, numerous researchers, including those involved in the initial study, have questioned diacetyl as a single causative agent [32,33,53,54,55,46]. Single researchers have also offered up contrasting views. Lockey and others [55] identified a NOAEL of 0.07 ppm and a LOAEL of 0.35 ppm based upon their work. However, the same authors [55] appeared cautious in linking diacetyl to disease occurrence, owing to the fact that most studies only provide measures of cumulative exposure, with little specific data exploring the impact of exposure duration on disease occurrence. For comparison, Maier and others [56] justified an OEL recommendation of 0.20 ppm diacetyl vapor as an 8-h TWA, primarily derived from mice data reported in Morgan and others [33]. More recently, a re-analysis was conducted on the Health Hazard Evaluation that was performed by NIOSH (123) same spirometry results and employment histories) regarding the pulmonary status of workers at the flavorings manufacturing factory, to account for inherent bias (the fact that pulmonary health data are inherently correlated as a result of the longitudinal nature of spirometry testing, Ronk and others 2013). The researchers concluded that exposure to flavoring compounds, such as diacetyl, at work did not raise the probability of aberrant spirometric data. (Diacetyl in Foods: A Review of Safety and Sensory Characteristics. Comprehensive Reviews in Food Science and Food Safety 14:5, 634-643 Online publication date: 1-Sep-2015)."

3.2 Tobacco Studies Demonstrate that Diacetyl is Not the Cause of Bronchiolitis Obliterans

There are currently 1.22 billion smokers worldwide, and since the Surgeon General's first warning of health risk in 1964-65, over 300 million deaths globally have been the direct result of cigarette smoking's adverse health impacts. Tobacco studies represent the largest global public health epidemiological investigation in human history. Thousands of articles (over 7000 were reviewed for the Surgeon General's 1964 Report and over a trillion dollars have been spent studying adverse health effects associated with smoke inhalation from consumer cigarettes, cigars, and pipes. It has been known since the 1960s that tobacco contains diacetyl [57] and that the smoke emitted contains diacetyl since 1981[58].

There are approximately 600 ingredients in cigarettes, including diacetyl. When burned, they generate more than 7,000 chemicals, including diacetyl. At least 69 of these chemicals are known to cause cancer, and many are poisonous [59]. In 2011, the tobacco industry established a list of harmful and potentially harmful constituents (HPHCs) in tobacco products and tobacco smoke (the established HPHC list via the Federal Register) consistent with requirements of the Federal Food, Drug, and Cosmetic Act (the FD&C Act). The established list of 93 HPHCs is included in the notice. Diacetyl was not included as a harmful ingredient (even though it is a known component in both cigarettes and cigarette smoke).

The 2014 Surgeon General's report on smoking identified the primary lung diseases associated with tobacco use to be chronic obstructive pulmonary disease (COPD), asthma, lung cancers and mycobacterium tuberculosis. BO was not mentioned as a smoking-related disorder.
(http://www.surgeongeneral.gov/library/reports/50-years-of-progress/full-report.pdf)

The level of diacetyl a smoker is exposed to in cigarettes with natural or flavored tobaccos is approximately 100 times the highest levels of diacetyl reportedly measured in the Jasper GML Plant air samples from 2000-2003 by NIOSH (see Fig. 1).

In addition, the diacetyl in a cigarette is inhaled directly into the lungs (intake vs dose). In contrast, plant workers would inhale diacetyl only if it was present in ambient air at the point of exposure. None of over 50 years of published literature on health risks associated with smoking has reported a single verified case of BO associated with cigarettes, cigars, or pipes.

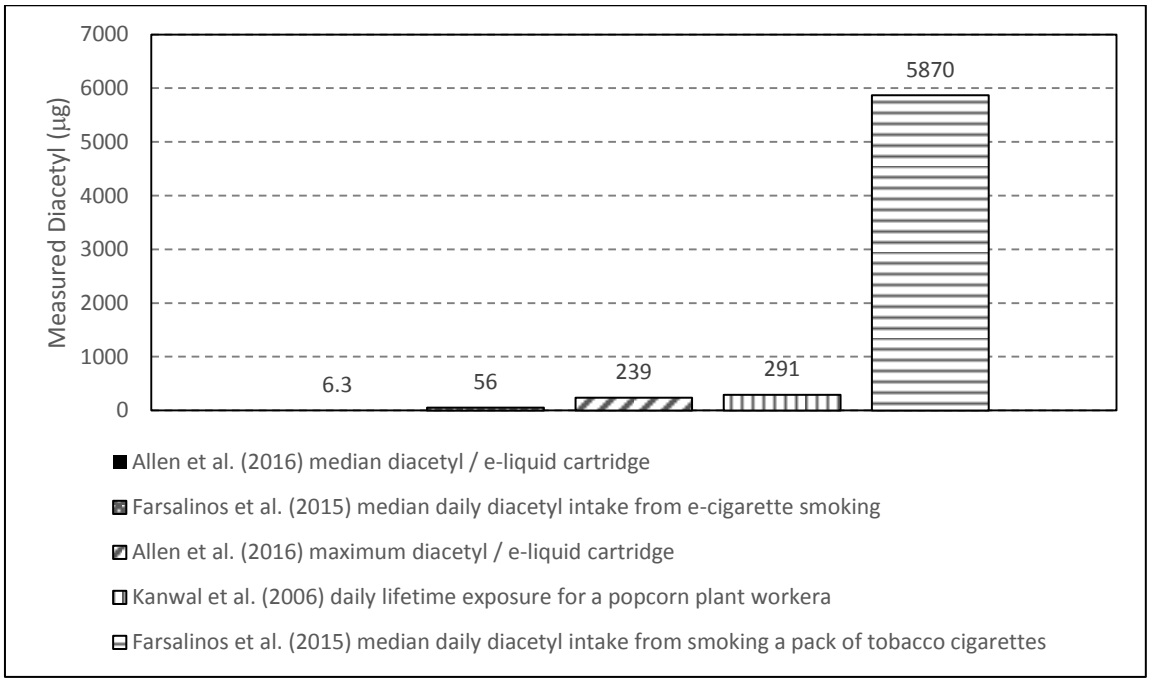

Fig. 1. Diacetyl inhalation - butter flavoring vs. cigarette smoking

[a] Diacetyl exposure (µg) calculated as follows: 1 ppm diacetyl = 3.55 mg/m3m, thus 200 ppb diacetyl = 0.71 mg/m3. 0.71 mg/m3 x 10 m3 of inhaled air breathed in an 8-hour shift = 7.1 mg (7100 µg) inhaled diacetyl per shift. The average dose over 365 days/year for 40 years = 7100 µg x 200/365 (working days) x 3/40 (working years) = equivalent daily lifetime dose of 291 µg/day

Cigarette smoking is known to be the predominant global cause of chronic bronchitis, emphysema, respiratory bronchiolitis, and respiratory bronchiolitis-associated interstitial lung disease. Respiratory bronchiolitis and respiratory bronchiolitis-associated interstitial lung disease are associated with mild fibrotic peribronchiolar changes; furthermore, chronic bronchitis and emphysema can progress to concurrently involve BO lesions, but this does not meet the clinical definition of BO disease [60].

In the Final Diacetyl Recommended Exposure Level [61] document NIOSH discussed a recent study addressing diacetyl measured in cigarette smoke in the context of whether smoking-related exposures might contribute to incidence of BO. Researchers collected mainstream cigarette smoke via a smoking machine and reported presence of diacetyl for seven types of cigarettes, with the average level ranging between 285 µg diacetyl/cigarette (250 ppm) for the International Organization for Standardization (ISO) parameters and 778 µg diacetyl/cigarette (361 ppm) by the Health Canada Intensive (HCI) parameters [45]. In one study the total mainstream smoke withdrawn from each burning cigarette from 15 different commercial reference cigarettes was analyzed by GC-MS and reported a range of 301–433 µg diacetyl/cigarette [62]. Another study utilized a smoking machine to evaluate potentially inhaled smoke from 41 different types of cigarettes, GC/MS for analysis with ISO parameters, and reported a measured range of 12.7–145 µg diacetyl/cigarette [63]. NIOSH also reported that electronic cigarette liquids often contain diacetyl [64,65].

NIOSH cited the Pierce et al. [45] report as the first study to report calculated diacetyl concentrations in mainstream cigarette smoke in ppm but argued that the reported estimates as measured using ISO parameters are not actually comparable to workplace exposures, considering that inhaled mainstream smoke is not the only source of breathed air. NIOSH argued that, assuming that the other air breathed by smokers does not contain diacetyl, the workplace equivalent exposure concentration for a smoker during the smoking time period would be roughly 190- to 560- fold lower than the concentration measured in the mainstream cigarette smoke using the ISO parameters (one 0.035 L puff/min - 6.75/0.035 is 193 and 19.5/.035 is 557). NIOSH further considered differences in breathing patterns among cigarette smokers or electronic

cigarette users (“vapers”) and employees - which have the potential to affect the pharmacokinetics of inhaled diacetyl [66] – and generated lower estimates of inhaled diacetyl concentrations, still asserting that such levels would be predicted to decrease FEV1 in some individuals if inhaled for a working lifetime [61]. NIOSH cited the observation that many smokers do demonstrate significant decreases in FEV1 [67,68,69,55,129] adding that cigarette smoking is a major risk factor for chronic obstructive pulmonary disease (COPD) for which decreases in FEV1 are a characteristic feature.

NIOSH also pointed out that bronchiolar fibrosis is part of the airway remodeling response characteristic of COPD [70,71], attempting to draw a connection to BO, which is also typified by decreases in FEV1 and fibrosis of bronchioles [72]. NIOSH acknowledged, however, that smokers with COPD have additional morphologic pulmonary changes, including emphysema, that are not seen in BO [73]. Smoking-related interstitial fibrosis (SRIF) is a common and morphologically unique feature in smokers' lung tissue (124,129). On the basis of histology, SRIF can be separated from idiopathic interstitial pneumonias and other types of pulmonary interstitial fibrosis [74]. SRIF causes dense thickening of the alveolar septa by thick collagen bundles with a hyalinized appearance, often with the admixture of bands of hyperplastic smooth muscle. There is little concomitant inflammation. SRIF is most common in the subpleural and centrilobular parenchyma and is frequently associated with centrilobular emphysema and respiratory bronchiolitis. The majority of SRIF patients do not exhibit clinical symptoms [75].

Finally, NIOSH considered the hypothesis that the failure to diagnose diacetyl-induced BO as a cigarette smoker-associated disease suggests that diacetyl does not cause BO in exposed employees [45] and argued that the data (as re-evaluated according to NIOSH’s own assumptions corresponding to occupational exposure concentrations) may instead suggest the hypothesis that diacetyl and related reactive carbonyl compounds in cigarettes could potentially contribute to COPD and should be further studied. The agency concluded by asserting that the presence of diacetyl in cigarette smoke does not diminish the need to control workplace exposures to diacetyl [61].

The concentrations purported by NIOSH to be occupational levels of diacetyl are derived from pre-2008 retrospective studies that used a never validated, irreproducible, inaccurate and non-correctable (in mixtures as per Cox-Ganser, [50]) NIOSH Method 2557 and are therefore unreliable. The diacetyl concentrations reported by Pierce et al. [45] are not the concentration solely in tobacco smoke but the cigarette itself - concentrations increase with burning/ignition. In addition, the NIOSH’s lower estimates of diacetyl exposure due to assumptions of smoking duration should be reconsidered. The average smoking duration is over a 24-hour day, 7 days per week (75-180 minutes over 24 hours per day; Zacny and Stitzer, 1996) for 20-40 years at 365 days per year of exposure as compared to approximately 3 years of 8-hour days at a frequency of about 250 working days per year for the average flavoring plant mixer studied in the 5 plants reported by NIOSH between 2000-2006. Ventilation rate comparisons are of limited use as 24-hour exposures would entail both occupational and non-occupational measurements (including routine exercise). Diacetyl levels as measured by NIOSH in the study of the 5 plants from 2000-2006 (using the flawed NIOSH Method 2557) represent potential exposure point concentrations and not doses inhaled by workers. Smoking machines, however, measure inhaled doses so direct comparisons of diacetyl concentrations generated by these two measures are invalid. To that point secondhand smoke studies have demonstrated that between puffs a smoker still inhales an injury causing concentration of toxic mixtures at approximately 1/3 the concentration of the actual puffing dose. Interestingly in the 6 years since publication of the NIOSH REL not a single study has produced a case of BO attributable to tobacco smoking despite thousands of physician visits, case studies and research publications on smoking related lung injury. Smoking-related interstitial fibrosis and COPD are different than BO by etiology, structure and radiological features. To continue to ignore large, diverse data sets on smoking and lung injury is not in the interest of public health protection (Mink and Ungers, [76], NIOSH Diacetyl Draft Comments Docket). Some argue that regulating ‘marker’ or associated airborne contaminates is a conservative approach- but it is exactly the opposite. In failing to define a causative agent, or defining the wrong causative agent, in occupational exposures to complex mixtures worker protections cannot be appropriately developed. The reduction of all 120 VOCs at the Jasper, Missouri, GML plant in 2002 (as well as the other 1000

chemicals/particulates/oils) primarily by plant-wide engineered controls resulted in dramatic reductions in lung function declines. Yet these effective, broad exposure reductions did not define the causative agent/s. That agent or agents has yet to be identified.

Billions of smokers have inhaled approximately 300 ppm of diacetyl daily by direct inhalation while being studied by tens of thousands of physicians, and scientists globally without a single recorded case of BO (smoking has been linked to lung diseases since the 1940s, Surgeon General's Report 2014). Also noteworthy is the fact that as early as 2002 pediatric pulmonology studies recognized that smoking in particular was not a risk factor in the development of BO through in utero or secondhand exposures [77,78,79,125]. The human data are overwhelming with respect to support for the position that failure to diagnose BO as a smoking-related illness casts serious doubt on diacetyl as a causative agent.

3.3 Literature Evaluation through the lens of Hill's Aspects of Association

To assist his occupational medicine colleagues in evaluating observed associations between exposure and disease, Hill [80] proposed nine "aspects of. . . association" (also commonly referred to as "criteria") that should be taken into consideration before concluding that the most likely interpretation of an association is causation (strength of association, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment, and analogy) [81]. The following is a discussion of the scientific evidence regarding the reported association of obstructive lung disease and exposure to diacetyl and diacetyl-containing butter flavorings in light of the Hill criteria (see refs. 82-107).

3.3.1 Strength of association – The larger the association between exposure and disease, the greater the likelihood of causality

The strength of the reported association between exposure to diacetyl and/or diacetyl-containing butter flavorings should be considered in light of several factors. The nature of the observed lung conditions reported in the early NIOSH studies varies; the pathophysiology of the reported obstructive lung conditions has not been established; and there were other suspected causative agents of BO present in the Jasper GML Plant that were not ruled out (e.g., Akpinar-Elci et al., [35]). Additionally, the underlying data collected in the Jasper GML Plant investigation (and all subsequent diacetyl-focused epidemiological workplace investigations through 2008) should be viewed in light of the revelation of the weaknesses and reliability issues of the NIOSH Method 2557 used to generate the diacetyl exposure data. The validity of the suspected association is also confounded by absence of pre-employment lung function tests or histories for the reported cases, and lack of knowledge regarding prior or concurrent occupational or personal exposures to other known or potentially causal agents associated with obstructive lung disease and BO.

3.3.2 Consistency – is demonstrated by association between two variables, with respect to the null hypothesis, across multiple epidemiological studies with varying locations, populations, and methods

In reviewing NIOSH reports of occupationally related obstructive lung disease at popcorn plants [39], the lack of pre-employment clinical data and the variation in the signs, symptoms and diagnostic findings of the observed lung disease cases suggests that they do not represent repetitive observation by different persons in different places, circumstances and times of a consistent association between diacetyl exposure and obstructive lung disease. Some have interpreted the NIOSH findings that diacetyl is a cause of BO and other lung diseases among workers exposed to diacetyl have been confirmed by epidemiological studies performed by other researchers [55,82,20,21,43]. These studies, however, are also impacted by the analytical Method 2557's inability to reliably and accurately measure airborne diacetyl in the plant environment. Without consideration of pre- or outside employment exposures, variable diagnostic protocols, and potentially inaccurate underlying data in all of the sentinel studies and ignoring all other compounds workers were co-exposed to (including those associated with BO development), the criterion of consistency remains unsupported.

3.3.3 Specificity – Associations are more likely to reflect causality if/when they are specific (i.e., the observed exposure only causes one disease)

There is no specificity from which to conclude that diacetyl or diacetyl-containing butter

flavorings are the cause of obstructive lung disease and/or BO. There are other known causes of BO that the literature does not address. Kanwal's [51] retrospective analysis of the 2002 Jasper GML Plant reported that reduction in diacetyl exposure results in marked improvement in respiratory symptoms and lung function among plant employees. This has been cited as support for a specific relationship between diacetyl and lung disease in spite of the fact that potential exposures to essentially all airborne chemicals were significantly reduced by the engineering controls implemented [54,126].

3.3.4 Temporality – A conclusion of causality requires that exposure precede the incidence of disease

Without critical pre-employment lung function data for reported obstructive lung disease cases in plant workers allegedly exposed to diacetyl, definitive conclusions regarding temporality are difficult if not impossible to make. The reported industrial hygiene studies did not have pre-employment lung function testing and none reliably measured diacetyl exposures or accounted for confounding co-exposures.

3.3.5 Biological gradient (dose-response) – Causality requires the observation of a dose-response relationship between the observed exposure and disease

The literature contains insufficient information to reliably conclude that there is a dose-response relationship between exposure to diacetyl or butter flavors containing diacetyl and obstructive lung disease or BO. An evaluation of the epidemiology studies as a whole shows no consistent relationship between the duration and/or magnitude of diacetyl exposure and increased incidence of lung disease [20,21,127].

3.3.6 (Biological) Plausibility – This criterion is satisfied if the observed association is consistent with body of evidence regarding etiology and the mechanism of disease

The results of animal studies that have been performed on diacetyl or butter flavors containing diacetyl cannot be extrapolated to humans because, among other things, they are unsupported by reproducible epidemiological data with known concentrations of Diacetyl and relevant pre-employment exposure data.

3.3.7 Coherence – The explanation of association and causality should make sense in light of the current state of the science and knowledge available to the researcher

The literature is insufficiently coherent to support a conclusion that exposure to diacetyl or butter flavorings containing diacetyl can cause obstructive lung disease or BO. The notion that exposure to diacetyl or butter flavors containing diacetyl causes BO conflicts with the well-established studies in humans, including those of cigarette smokers. In fact, literature reporting on thousands of properly conducted studies unequivocally shows that exposure to diacetyl does not cause BO. Furthermore, the findings of the animal studies performed to date cannot be extrapolated to humans because, among other things, they are unsupported by epidemiological data. Some researchers have suggested that diacetyl causes significant inflammatory markers (neutrophilic airway inflammation) consistent with the clinical findings of bronchiolitis obliterans and other lung diseases [37]. However, neutrophilic airway inflammation is consistent with most lung irritations, the most common being the common cold.

3.3.8 Experiment - Hill suggested that, when possible, investigators should appeal to experimental or semi-experimental evidence in their analysis of the association in question

The example that Hill provided was the observation of preventive measures implemented following the detection of an association, and subsequent efforts to answer whether the measures actually prevent the disease outcome. The only potentially relevant evidence on this criterion consists of efforts at the Jasper GML Plant to reduce all potential exposures in the plant by improving engineering controls. However, since those efforts reduced exposure to all volatile organic compounds, dust, salt, oil and other substances in the plant, some of which are known causes of BO, it is not reasonable to draw any conclusions regarding a correlation between diacetyl or butter flavors containing diacetyl and the reported state of lung conditions following implementation of those efforts.

3.3.9 Analogy

Hill suggested that when evidence is strong for a causal relationship between a specific agent and disease, investigators should be more open to

accepting weaker evidence of such an exposure-response relationship for a similar agent. There are a number of toxic substances that are recognized in the scientific and medical literature as known causes of obstructive lung disease and BO. Diacetyl is not structurally similar to any of those substances. The modern value of the analogy concept is gained through the proposal and testing of mechanistic hypotheses rather than from confirming causal inferences among multiple potential causal agents [81]. Perhaps the strongest mechanistic evidence and argument put forth from researchers into the proposed relationship between diacetyl and obstructive lung disease and/or BO is the finding from the work of Hubbs et al. [19]. These researchers treated three groups of mice with a one-time dose of 100, 200, and 400 mg/kg bw/day, respectively, administered by oropharyngeal aspiration. They reported fibrohistiocytic proliferation at the junction of the terminal bronchiole and alveolar duct for the mice in the 400 mg/kg bw/day dose group. Hubbs et al. suggested that this was possibly representative of early stages of BO and might be relevant to lower airways in the human. These study results and associated inferences are confounded by the physiologically irrelevant route of administration. Oropharyngeal aspiration is a known cause of BO from transplant studies rendering the Hubbs et al. [19] results of limited utility due to confounding bias [128].

BO is a rare disease involving concentric bronchiolar fibrosis known to rapidly develop in cases of acute inhalation exposure to select irritant gases at sufficiently high concentrations. Risk of developing BO from exposure to irritant gases is substantially driven by toxicologically relevant concentrations of the gases occurring at the site of bronchiolar epithelium. Highly soluble irritant gases such as ammonia that cause BO generally follow a threshold-dependent mechanism of action involving cytotoxicity and, at sufficiently high exposures, these gases result in severe inflammation of the upper respiratory tract along with concurrent inflammation of the bronchiolar epithelium. Acute respiratory distress follows, along with pulmonary edema, and post inflammatory concentric fibrosis that become clinically obvious typically within a few months. To date, animal and human studies focused on airborne diacetyl, which is an example of a highly soluble gas, have not identified a coherent pattern of pathology and latency that would be expected based upon studies of other known causes of BO. Also, studies to date have not unequivocally identified strong, specific and coherent toxicological mechanisms that would advance the understanding of allegedly diacetyl-related respiratory effects at exposure concentrations relevant to humans, occupational or otherwise [60]. In toto there is no similar agent studied to date with a biological analogous pattern of lung insult in humans at concentrations found in ambient or indoor air including highly complex flavoring manufacturing sites. More recent studies in coffee grinding facilities are confounded by different analytical precision from previous studies as well as the presence of plant oil emissions a suspected causative agent of BO.

4. CONCLUSION

Evidence presented in the scientific and medical literature to date is insufficient to support a conclusion of causality between exposure to diacetyl and incidence of bronchiolitis obliterans or obstructive lung disease. Results of retrospective occupational exposure studies at flavoring/popcorn plants are inadequate to confirm causality of diacetyl with respect to reported lung conditions in the worker population due to several deficiencies: insufficient characterization of all of chemical identity and accurate airborne concentrations of the substances, including diacetyl, to which the employees were potentially exposed both inside and outside the workplace; and the absence of pre-employment lung function data. NIOSH Method 2557, which is the air sampling method used to generate that data underlying the current regulatory literature and scientific findings, is unreliable and inappropriate for diacetyl detection or quantification, as confirmed by NIOSH.

Large, long-term, reliable, relevant and reproducible human epidemiological studies of cigarettes offer strong support for the conclusion that diacetyl does not cause BO in humans. Cigarette smoke has been shown to contain diacetyl at levels hundreds of times higher than that found in reported occupationally related flavoring plant exposures and are directly inhaled versus ambient air exposures in occupational exposures. Global tobacco smoking health data since the 1940s, reflecting diacetyl exposure by billions of smokers, has not shown a single case of BO. It is likely that complex mixtures of heated salt particles saturated with soy based plant oils and other organic vapors (over 1000 in many flavoring plants) were potentially causative of chronic lung disease in occupational environments before better engineering controls

(i.e better ventilation, etc.…) were instituted widely two decades ago. Diacetyl's statistical association with BO and other chronic lower airway disease at concentrations found in any known occupational setting studied to date appears to be a function of flawed analytical procedures and subsequent data analysis. Diacetyl like all chemical compounds is a hazard at some dose (i.e "the dose makes the poison") but not at concentrations found at or below those in common tobacco product's smoke (45,129).

COMPETING INTERESTS

Author has declared that no competing interests exist.

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