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Recognizing occupational effects of diacetyl: What can we learn from this history?

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Abstract

For half of the 30-odd years that diacetyl-exposed workers have developed disabling lung disease, obliterative bronchiolitis was unrecognized as an occupational risk. Delays in its recognition as an occupational lung disease are attributable to the absence of a work-related temporal pattern of symptoms; failure to recognize clusters of cases; complexity of exposure environments; and absence of epidemiologic characterization of workforces giving rise to case clusters. Few physicians are familiar with this rare disease, and motivation to investigate the unknown requires familiarity with what is known and what is anomalous. In pursuit of the previously undescribed risk, investigators benefited greatly from multi-disciplinary collaboration, in this case including physicians, epidemiologists, environmental scientists, toxicologists, industry representatives, and worker advocates. In the 15 years since obliterative bronchiolitis was described in microwave popcorn workers, α-dicarbonyl-related lung disease has been found in flavoring manufacturing workers, other food production workers, diacetyl manufacturing workers, and coffee production workers, alongside case reports in other industries. Within the field of occupational health, impacts include new ventures in public health surveillance, attention to spirometry quality for serial measurements, identifying other indolent causes of obliterative bronchiolitis apart from accidental over-exposures, and broadening the spectrum of diagnostic abnormalities in the disease. Within toxicology, impacts include new attention to appropriate animal models of obliterative bronchiolitis, pertinence of computational fluid dynamic-physiologically based pharmacokinetic modeling, and contributions to mechanistic understanding of respiratory epithelial necrosis, airway fibrosis, and central nervous system effects. In these continuing efforts, collaboration between laboratory scientists, clinicians, occupational public health practitioners in government and industry, and employers remains critical for improving the health of workers inhaling volatile α-dicarbonyl compounds.

Keywords

2,3-Butanedione; 2,3-Pentanedione; Obliterative bronchiolitis; Flavor; Diacetyl

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None.

Disclaimer

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1. Introduction

The discovery of diacetyl-related obliterative bronchiolitis illustrates many steps in the recognition and control of emerging occupational lung diseases in the twenty-first century. The specifics of this discovery may have generalizable lessons for all of us concerned with protection of workers. These lessons are especially important given the current pace of new introductions of new and old chemicals with workplace inhalation exposures. Although the toxicity of diacetyl to workers was not suspected until late 2000, cases retrospectively attributed to diacetyl occurred at least 15 years earlier that even spurred toxicology experiments in the chemical industry. Thus case clusters of obliterative bronchiolitis were recognized in two flavoring manufacturing companies in 1985 (NIOSH, 1986) and between about 1993 to 1996 (Lockey et al., 2002), but the mixed and varying flavoring ingredient exposures precluded identification of a single causal exposure. Nonetheless, in 1993, a chemical manufacturer exposed rats to diacetyl, documented lethality after inhalation exposure, and confidentially reported findings to the flavoring industry trade association [BASF, 1993]. Both case clusters resulted in employee litigation against the flavoring company employers. The Flavoring and Extract Manufacturing Association conducted an educational meeting about types of occupational lung disease for its members in 1997. Emphasis on obliterative bronchiolitis cases was not evident, but diacetyl causality would have been unclear at that time, because the cases had many chemical exposures. The National Institute for Occupational Safety and Health (NIOSH) investigators received the German animal laboratory studies from the medical director in 2003 when the agency became aware of their existence, having already initiated its own animal studies. The lesson here may be that surveillance for emerging occupational hazards might be facilitated by tracking litigation and by exchange of proprietary science among parties concerned with prevention.

2. Microwave popcorn manufacturing risk

Occupational lung disease risk in the microwave popcorn industry remained unsuspected for many years because affected workers had no work-related pattern of symptom exacerbation and no evident toxic exposures. In fact, the obliterative bronchiolitis diagnosis affecting workers remained unrecognized because most physicians associated this disease with a history of overwhelming exposure causing acute pulmonary edema, followed by evolution of fixed airflow limitation in the weeks following apparent recovery. Historically, this dramatic prelude to disease presentation was the only clinical means of attributing an individual case of obliterative bronchiolitis to an occupational or environmental exposure. Thus, affected former workers in the sentinel microwave popcorn plant in Missouri received physician misdiagnoses of asthma, emphysema, and bronchitis, all common diseases that did not arouse concern for an occupational etiology. Indeed, the severity of lung disease in 8 former workers resulted in 4 being listed for lung transplant, and a local pulmonologist reported his concern about severe disease in young nonsmokers to the Occupational Safety and Health Administration (OSHA). Understandably, this government enforcement agency only measures regulated exposures and hence rarely pursues emerging hazards. OSHA reported no recognized lung hazards in the microwave popcorn plant to the pulmonologist. The

lesson here is that public health resources (rather than a regulatory agency) might have been a more promising route of investigation of an unsuspected hazard. But the key to mobilizing public health resources to bear was the occurrence of a case cluster. The 8 former workers with very severe lung disease indicated a statistically unlikely occurrence in a plant with a current employment of about 130 employees [CDC, 2002]. While isolated individual cases might be difficult to attribute to a workplace exposure, epidemiologic tools can identify exposures or work processes that conferred excess risk of lung disease. Epidemiologic tools are only pertinent in the context of a cluster of cases and systematic collection of information on a population at risk such as a workforce. Had the 8 sentinel cases occurred in a major metropolitan area served by many medical specialists, the cluster might never have been recognized, and search for an etiology would not have occurred.

Suspicion of diacetyl, however, had not occurred with the two earlier clusters of cases in flavoring manufacturing. A necessary condition for recognition was a simpler exposure environment, as occurred in microwave popcorn manufacture. Further, recognition of a new occupational risk required understanding that we were not looking for a known chemical hazard. The NIOSH environmental team lost valuable time by assaying for air concentrations of particulate matter because clouds of microfine salt had obscured visibility in the plant historically, even though salt exposures, such as in mining, were an implausible cause of an irreversible lung disease. Similarly, bioaerosols, such as endotoxin, had decades of study without having been associated with obliterative bronchiolitis. Volatile components of flavorings for microwave popcorn were low enough on the list of environmentalists' suspicion that personal breathing zone samples were not obtained by job title in the first cross-sectional study of the microwave popcorn plant workforce, with area samples being obtained instead. Physician interviews of sentinel former worker cases and current workers supported a volatile etiology, rather than particulate etiology, because mixers of heated flavoring in oil were disproportionately affected among the former workers. In addition, workers reported frequent mucous membrane irritation requiring eye drops, particularly with the use of "light" flavoring formulations which had higher concentrations of butter flavors in less oil. Enhanced multidisciplinary exchange in planning between physicians and industrial hygienists might have been beneficial in both anticipating future regulatory needs for personal air sampling to support exposure-response analyses and in advising appropriate respiratory protection as the high prevalence of airflow limitation was being characterized. Nevertheless, the level of multidisciplinary interaction at NIOSH is much higher than in many organizations and was essential in identifying this new cause of disease. For emerging lung diseases, exchange of information and approach among disciplines is an extraordinarily valuable lesson.

Interdisciplinary exchange also benefits toxicologic approaches to controlled experimentation. An initial attempt at NIOSH to reproduce a complex laboratory chamber environment for rodents with mixed salt and flavorings was unsuccessful. Inhalation exposure to volatiles from one butter flavoring used in the sentinel microwave popcorn resulted in rodent respiratory deaths within the first few hours of exposure (Hubbs et al., 2002), allowing further epidemiologic and environmental studies and interventions to focus on flavorings. Toxicologists quickly proceeded to exposures to diacetyl, the predominant volatile in the flavoring mixture and in plant air (Hubbs et al., 2008; Morgan et al., 2008).

Single component exposure is always more readily interpretable with respect to biologic plausibility, even though some evidence later suggested that the presence of other volatile components in butter flavorings potentially contributed to the degree of toxicity associated with the flavoring formulation. An experimental basis for this evidence of mixed exposure effects has since evolved with the demonstration that butyric acid affects diacetyl binding and metabolism (Morris and Hubbs 2009) and with studies of 2,3-pentanedione, a diacetyl analog with a fifth carbon, with similar toxicity (Hubbs et al., 2012; Morgan et al., 2012a,b).

In exploring emerging issues, no substitute exists for an appreciation of what is known, the limits on that knowledge, and how new scientific and medical knowledge emerges. The discovery of flavoring-related lung disease illustrates many challenges in recognizing what is known and unknown. No risk of obliterative bronchiolitis associated with food production was widely known outside of a couple of flavoring companies and the trade association in 2000. The sentinel cases did not share one clear diagnosis and did not fit the textbook understanding of chemically-induced obliterative bronchiolitis based on individual cases following dramatic, usually accidental, overexposures. Obstructive spirometric abnormalities, commonly attributed to cigarette smoking, rarely occur in smokers before middle age [Wise, 2008], and the sentinel cases included mostly young persons and nonsmokers (Akpinar-Elci et al., 2004). The cases did not respond to medical treatment for asthma or other common lung diseases such as bronchitis and emphysema. The company management relied on Food and Drug Administration designation of flavoring chemicals as “’generally recognized as safe” for ingestion, without considering a possible inhalation hazard that had not been previously described. As the toxicology community knows well, evaluation of inhalation toxicity is expensive and rarely undertaken in the absence of commonly recognized sequelae following accidents or study of chemical warfare agents. In fact, the dearth of inhalation toxicology testing of common chemical exposures may obscure many potential causes of short latency, irreversible airways disease that is not associated with acute incapacitation or exposure-related exacerbation of chest symptoms.

Eight severe cases of lung disease in former workers at one small rural food production factory was a compelling anomaly in what was known [CDC, 2002; Akpinar-Elci et al., 2004], as was a cross-sectional study of the current workforce that demonstrated that 25% of the employees had abnormal lung function (Kreiss et al., 2002). Epidemiology supported that diacetyl exposure was associated with risk of chest symptoms, abnormalities in lung function, and quantitative indices of lung function (Kreiss et al., 2002). This diacetyl association became biologically plausible as a cause with the conduct of animal exposure experiments over the succeeding months and years (Cichocki et al., 2014; Gloede et al., 2011; Goravanahally et al., 2014; Hubbs et al., 2014, 2002, 2012, 2013, 2008, 2010; Mathews et al., 2010; Morgan et al., 2008, 2012a, 2012b, 2015; Morris and Hubbs 2009; Palmer et al., 2011).

2.1. Limits of epidemiology

Epidemiology is an observational, rather than experimental, science but long-standing guidance exists for interpreting epidemiologic findings as causal. In the 1960s, Sir Austin Bradford Hill suggested that causality is more likely when the strength of association is

high; the findings are reproducible in diverse populations or by different investigators; the exposure precedes the effect; a dose- or exposure-response relationship exists; there is biologic plausibility; and that alternative explanations are unlikely (Hill 1965). In the case of the microwave popcorn investigation, we met the guidance for strength of association easily, since the never-smokers in the factory workforce had 10.8-fold the risk of airways obstruction compared to the general population of the same age and gender distribution (Kreiss et al., 2002). We also showed a relation between estimated cumulative diacetyl exposure (based on tenure in job-specific average exposures) and health indices from spirometry (Kreiss et al., 2002). For a rare disease, alternative explanations from known causes were easily ruled out for this population, although we did have to go through the exercise of examining state mortality from chronic obstructive lung diseases and smoking rates by county to meet the allegations of medical experts advising the company.

However, the other guidance for causal interpretation usually takes time to accumulate. Reproducibility required looking at other diacetyl-exposed workforces. With the assistance of state public health agencies, unions, and serendipity, we demonstrated that four of five other microwave popcorn plants had employee cases consistent with obliterative bronchiolitis (Kanwal et al., 2006). Other investigators reported sentinel cases in flavoring manufacturing plants [CDC, 2007; Hendrick, 2008], and we became aware of the two previous clusters of obliterative bronchiolitis in the flavoring industry (Lockey et al., 2002; NIOSH, 1986). We addressed temporality by showing that lowering diacetyl exposure halted the excessive decline in serial spirometry in the sentinel microwave popcorn factory (Kreiss, 2007) and that affected workers tended to stabilize their lung function with exposure cessation (without improvement, however) (Kanwal et al., 2011; Akpinar-Elci et al., 2004). Despite stabilization after exposure cessation, the disease is often serious and life-shortening. The 11-year follow up of the current and former workers tested by NIOSH in 2000–2003 showed a 4-fold excess of respiratory mortality from “other COPD” compared to national age- and gender-adjusted rates (Halldin et al., 2013). The iterative accumulation of evidence important in persuading the scientific community of the diacetyl hazard has a corollary: No single study or publication stands alone as the knowledge evolves, and the process of applying the accumulating evidence for prevention of suffering arising from workplace exposures is complex and slow, particularly in the setting of litigation.

2.2. Toxicologic confirmation of epidemiology

The experiments using α-dicarbonyls to cause respiratory epithelial necrosis and resulting obliteration of bronchioles are perhaps the most persuasive of the factors considered in evaluating epidemiologic associations as causal. This toxicologic body of evidence in animals has taken more than a decade to accumulate, is still evolving, and in turn is prompting epidemiologic hypotheses to be investigated in human populations. First, diacetyl exposures were demonstrated to result in exposure-dependent sloughing of nasal respiratory epithelium in mice and rats after acute exposure (Hubbs et al., 2002, 2008; Morgan et al., 2008). In response to concerns regarding the translational relevance of epithelial necrosis in rodents to bronchiolar effects in humans, hybrid computational fluid dynamic-physiologically based pharmacokinetic modeling showed that nasal scrubbing of diacetyl precluded equivalent concentrations to rodent bronchiolar epithelium (Morris and Hubbs

2009; Gloede et al., 2011). Bypassing the rat nose by intratracheal instillation demonstrated fibrosis and bronchial and bronchiolar effects (Palmer et al., 2011). Unrelated airway irritants, such as sulfur mustard, produce analogous nasal epithelial desquamation in rodents and obliterative bronchiolitis in humans (Ghanei et al., 2004, 2008; Rowell et al., 2009; Weber et al., 2010).

Flavoring manufacturers were quick to substitute 2,3-pentanedione for diacetyl as food producers demanded diacetyl-free flavorings, although the client food producers were not informed of the new formulations, protected as trade secrets (Day et al., 2011). Again, toxicologists required time to investigate the potential toxicity of 2,3-pentanedione in animal models (Morgan et al., 2012a,b; Hubbs et al., 2012). The substitute appeared to be as hazardous as diacetyl, although human data are never likely to be available for workers exposed uniquely to one or the other flavoring constituent. The actual mechanism of epithelial necrosis and respiratory fibrosis is opening up new areas of toxicologic understanding pertinent to protein damage, ubiquitination, cellular adhesion, gene expression (Morgan et al., 2015), and neuronal damage in the olfactory bulb (Hubbs et al., 2012, 2014). The last is ripe for investigation of olfactory effects in worker populations exposed to diacetyl. In addition, there is increasing exchange between toxicologists and those studying bronchiolitis obliterans syndrome occurring in the majority of organ transplant recipients.

Ironically, a study demonstrating the presence of diacetyl and 2,3-pentanedione in cigarette smoke was initially interpreted as providing evidence suggesting a need to question the scientific basis for the NIOSH draft recommendations for diacetyl and 2,3-pentanedione exposure limits (Pierce et al., 2014). However, smoking is a leading cause of chronic obstructive lung disease which shares critical morphologic characteristics with obliterative bronchiolitis, including airway fibrosis (Kim et al., 2008; Sohal et al., 2013). The NIOSH draft occupational exposure limits were based upon obstruction, and occupational diacetyl exposure is associated with obstruction after control for cigarette smoking status [NIOSH, 2011]. Given the strength of the epidemiology and toxicology studies linking diacetyl and 2,3-pentanedione with obstructive lung disease, the critical finding in the Pierce et al. publication is that α-dicarbonyl exposures from cigarette smoke are potential contributory factors leading to chronic obstructive pulmonary disease in smokers.

The controversy evident in interpretation of single human epidemiologic publications was initially duplicated in the toxicologic literature, likely motivated by the high economic stakes surrounding litigation and potential regulation. Delays in experimental progress have occurred due to the unavailability of BASF toxicology from the early 1990s in the published literature and arguments including that rodent models of obliterative bronchiolitis from α-dicarbonyls do not simulate human disease; that diacetyl content of burning cigarettes in a smoking machine was not reflected in obliterative bronchiolitis in smokers (Pierce et al., 2014); and that “natural” diacetyl and 2,3-pentanedione are harmless.

3. Flavor manufacturing risk in California

While industry-wide risk of obliterative bronchiolitis was being established in five of six microwave popcorn plants, cases were recognized in flavoring manufacturing workforces, both retroactively and contemporaneously. Attribution to diacetyl or 2,3-pentanedione in such workforces was impossible in individual cases, given the multiplicity of volatile exposures, but α-dicarbonyl compounds were usually present, as had been the case in both the 1985 NIOSH health hazard evaluation [NIOSH, 1986] and 1993–1996 flavor manufacturing cases (Lockey et al., 2002). Two case reports in flavoring manufacturing workers in California [CDC, 2007] resulted in collaboration of the state labor regulatory agency (CalOSHA) and the California Department of Public Health to prevent further flavoring-related obliterative bronchiolitis by health surveillance and environmental consultation. No easy means of identifying flavor manufacturers or diacetyl users existed at the time in California, a situation that has been partially rectified by a unique California regulation established in 2014 that manufacturers with toxic substances share information about shipping, usage, and toxicity [California Code of Regulations, 2016]. In exchange for exemption from compliance investigations by CalOSHA, 16 companies conducted questionnaire and spirometry health surveillance on flavoring-exposed workers and reported it to the state public health department (Kim et al., 2010). CalOSHA advised companies on engineering controls and respiratory protection. At the time of this governmental prevention program from 2006 to 2009, guidance for protective exposure limits was unavailable.

In this early public health prevention effort, compliance with health reporting requirements was spotty, as was coordination between the two state agencies. Nevertheless, outcomes included identification of four 4-person clusters of likely obliterative bronchiolitis among the companies that used the most diacetyl in manufacturing (Kim et al., 2010). While this finding reinforced the hazard of diacetyl use, no quantitative exposure data had been systematically collected with which to advance guidance for protective levels of exposure. Because flavors were “generally recognized as safe” for ingestion, these companies generally had given little attention to exhaust ventilation, isolation of flavoring sources, or respiratory protection for inhalation exposures.

The newly introduced medical surveillance of workers was more form than content, since the spirometry quality available from commercial medical providers was inadequate in 12 of 13 commercial providers (Kreiss et al., 2012). Even one university-based medical provider failed minimal spirometry quality criteria. As a result, the serial spirometry that would allow early identification of excessive pulmonary function deterioration during employment was limited to the few academic or government medical providers with excellent quality spirometry of flavoring workers. Cases of excessive spirometric decline clustered in the same four companies that had clusters of spirometric abnormalities consistent with obliterative bronchiolitis and high annual diacetyl usage in manufacture (Kreiss et al., 2012). This overlap of workforces with clusters of presumed cases, deterioration of pulmonary function during employment, and high diacetyl usage reinforced the hazard of diacetyl and also was proof of concept that evolving cases might be identified before irreversible abnormalities in lung function developed.

Many California medical providers did not use the questionnaire required by the state health authorities, so epidemiologic risk factors could not be examined for those workers. Some used OSHA respirator fit clearance questionnaires which do not collect job title, task, or symptom information. A provider recommended by the Flavor and Extract Manufacturing Association collected superb quality spirometry but used a different questionnaire for four companies and never reported systematic health findings in the medical literature that might have advanced the science. An industrial hygiene characterization of these companies was limited by use of an early NIOSH method for diacetyl that later required correction with absolute humidity, temperature, and days to extraction in the analytical laboratory [Cox-Ganser et al. 2011], which were unfortunately not collected (Martyny et al., 2008). In summary, the recognition of occupational lung disease in the “upstream” flavor manufacturing industry resulted in attention to exposures common to the “downstream” microwave popcorn industry, preventive collaboration between state public health and labor regulatory agencies, and experience that may prove valuable in future efforts including easier tracking of occupational hazards.

3.1. Other flavoring manufacturing epidemiology

Cross-sectional or longitudinal studies of other flavoring manufacturing work forces are sparse and limited to health indices. The difficulty of characterizing exposures in short-term batch operations producing many different flavors with different chemical ingredients precluded quantitative exposure-response analyses, although indices of exposure have supported the respiratory health hazard of flavoring manufacture. Two published investigations suggest differing risks across flavoring workplaces, as was found in California public health investigations. In a Kentucky flavoring plant with a high participation rate of workers in both production and office work, those with seven or more years of tenure had more shortness of breath and lower average measurements of lung function than those with less tenure, adjusted for age and smoking status; those spending an hour or more daily in production had excesses of many respiratory symptoms, lower average lung function, and more lung function abnormalities than those spending less time in production (Cummings et al., 2014). Insufficient quality of the company’s historical spirometry records precluded their use to assess longitudinal decline of lung function during employment.

The second flavoring company was distinguished by having company spirometry data demonstrating that about one-third of the production work force had abnormal spirometry (Kreiss, 2014). The pattern of spirometry abnormality, however, differed from that found in excess in the sentinel microwave popcorn plant in that almost all abnormalities were restrictive, rather than obstructive. Since this spirometric abnormality had not been emphasized in earlier flavoring-related publications, no concern for occupational lung disease was evident. When production workers were divided into two groups with greater potential for flavoring exposure (reflected by work area) compared to lesser potential for flavoring exposure, no difference in abnormal spirometry was evident in the two groups. However, the serial spirometry available from the company, limited to production workers, showed a striking difference in average and excessive decline in forced expiratory volume in one second between higher and lower potential exposure groups. The group with higher potential flavoring chemical exposure had 5.8-fold risk of excessive decline of lung function

during employment, compared to the group with lower potential for exposure. A contradictory industry-sponsored re-analysis of the same company data (Ronk et al., 2013) incorrectly described the lower exposure group as unexposed and omitted evidence of mean excessive decline in presentation of statistical models.

3.2. Restrictive spirometric abnormalities

The finding that 37% of the production workers of a flavoring manufacturing company had either abnormal restrictive spirometry or excessive decline in serial lung function was at first baffling, although work-relatedness was supported by excessive declines during employment and the nonuniform distribution of excessive decline by indices of exposure. Excessive declines, year after year, will eventually result in abnormal spirometry, as nearly one-third of production workers already demonstrated. The findings persisted when overweight and obesity were factored in, as well as change in weight over the spirometry testing period, age, ever-smoking, and tenure (Kreiss, 2014). The company neglected to ascertain what pathology was responsible for the excess of restrictive spirometric abnormalities. However, understanding of the clinical presentation of obliterative bronchiolitis has evolved markedly since flavoring investigations began in 2000.

The tip-off that obliterative bronchiolitis diagnoses were not limited to flavoring-exposed workers with fixed obstructive spirometry (not reversing with bronchodilators, as asthma usually does) came from a 2011 publication about obliterative bronchiolitis in U.S. soldiers returning from Iraq and Afghanistan (King et al., 2011). These soldiers had exertional shortness of breath that precluded their passing military requirements for a timed two-mile run, but the reason was not evident on spirometry, high resolution computerized tomography scans, or other common clinical diagnostic tests. Physicians at Vanderbilt University, seeing these soldiers in referral, took them to thoracoscopic biopsy, and most were found to have pathologic evidence of obliterative bronchiolitis. Of 38 with such pathology, spirometry was normal in 32 (84%); restrictive in 3; obstructive in 2; and mixed restrictive and obstructive in 1. Only 6 of 37 (16%) with pathologic obliterative bronchiolitis had air trapping on high resolution computerized tomography scan (King et al., 2011). Thus, usual diagnostic tests were insensitive for a diagnosis that precluded continued military service. Since that publication, others have come to light substantiating the range of spirometry found in pathologically demonstrable obliterative bronchiolitis. These include a hospital-based consecutive case series of obliterative bronchiolitis (Markopoulou et al., 2002) and internationally reviewed cases from mustard gas exposure in the Iran-Iraq war (Ghanei et al., 2008) that had defied diagnosis for two decades.

One take home lesson for the first decade of work on flavoring-related epidemiology is that limiting evaluated health outcomes to obstructive spirometry abnormalities likely created an underestimate of flavoring-related health effects. Indeed, in the sentinel microwave popcorn plant, the numbers of workers with pure restriction (N = 10), pure obstruction (N = 11), and mixed restriction and obstruction (N = 10) were nearly equivalent (Kreiss et al., 2002). We had lumped those with mixed restriction and obstruction with those with pure obstruction, assuming that air trapping related to obstruction accounted for the mixed spirometric picture. We now think that most of those with any spirometric abnormality may have had obliterative

bronchiolitis. The proportion of current production workers with exertional shortness of breath was 33%, higher than those with specific spirometric abnormalities. In retrospect, considering the U.S. soldiers with shortness of breath or the long-symptomatic survivors of mustard gas exposure in Iran, we neglected possible cases of flavoring-related occupational lung disease in those without spirometric abnormality. NIOSH investigators are currently reexamining historical popcorn plant data and follow up data to examine other clinical correlates and natural history (Cox-Ganser et al., 2016; Bailey et al., 2016).

4. Other industries with α-dicarbonyl exposure

Four cases consistent with obliterative bronchiolitis were found in a retrospective cohort of 103 process operators employed in manufacturing diacetyl in the Netherlands (van Rooy et al., 2007), as well as subclinical evidence of excessive chest symptoms and lowered pulmonary function in the entire cohort (van Rooy et al., 2009). Diacetyl is present in many natural products since it is a product of fermentation in dairy, wine, and beer industries and it is a product of roasting through the Maillard reaction, as in coffee. However, the recognition of occupational lung disease attributable to α-dicarbonyl exposure is scant. Exposure to butter flavoring in cookie dough manufacture resulted in a report of four cases from Brazil (Cavalcanti et al., 2012). Five severe cases were attributed to flavoring exposure in a coffee production plant (Bailey et al., 2015). However, the combined exposure to diacetyl and 2,3-pentanedione was as high in the area in which unflavored roasted coffee was ground as in the isolated flavoring room. Ongoing studies of unflavored coffee production explore health risks from the off-gassing of naturally produced diacetyl and 2,3-pentanedione during roasting and grinding of coffee. The absence of evidence of health risks from high levels of α-dicarbonyl exposure in industries, apart from microwave popcorn, cookie dough, flavoring, coffee, and diacetyl manufacture, may be a failure of recognition, as occurred in all of the industries in which cases were eventually recognized. The disease is rarely considered as a potential diagnosis in the absence of physician familiarity with obliterative bronchiolitis and occupational risks, symptoms temporally related to work, and acute onset from dramatic over-exposure. Usual diagnostic tests are insensitive, including spirometry and radiographic studies. Litigated cases have occurred in workers manufacturing flavored candy and snack foods, testing popcorn poppers, and popping microwave popcorn in video/DVD rental settings.

5. Socioeconomic context of stakeholders

Litigation surrounding this emerging health risk has had profound and predictable effects on the evolving science of α-dicarbonyl toxicity. Trade associations representing flavoring manufacturers and popcorn producers were helpful in disseminating information to members but sometimes held information that was not available to the entire scientific community. Collaboration between scientists and companies was inhibited by confidentiality agreements required of academic science consultants; limited information exchange from companies for possible legal reasons; and inconsistent access to worksites for government public health scientists to understand the industry. Legal defense used resources that might have been applied to preventive interventions. The practice of settling legal disputes without

acknowledging cause may confuse company personnel about responsibility to change conditions of work that resulted in worker hazard.

The five-year interval, between the 2011 NIOSH posting of a draft criteria document for recommended standards for diacetyl and 2,3-pentanedione for public comment [NIOSH, 2011] and its anticipated publication in 2016, reflects the difficulty of translating research findings into practice. While understanding the limitations of any single study is critical, acknowledging the iterative nature of scientific advance and the strength of converging lines of evidence from many disciplines and investigations is essential for timely worker protection. Government scientists try to respond to every public comment. The inefficiency and wasted resources that are inseparable from litigation delay guidance about safe levels and postpone prudent action for preventing more cases of ill health by engineering controls, respiratory protection, and worker education.

6. Toxicologic and public health outcomes

Despite delays, much has been accomplished over the last 15 years. An unsuspected area of mechanistic toxicology has opened up in exploring respiratory epithelial damage, its relation to protein damage, olfactory neuronal injury, pharmacokinetic modeling, altered gene expression during airway fibrosis, and possible relevance to transplant-related obliterative bronchiolitis and post-transplant restrictive lung disease (Cichocki et al., 2014; Gloede et al., 2011; Hubbs et al., 2012, 2013; Kelley et al., 2012; Kelly et al., 2014; Morris and Hubbs 2009; Morgan et al., 2015). Biomarkers for transplant-related obliterative bronchiolitis are being evaluated in occupational cohorts at risk for occupational lung disease. Within the occupational lung disease community, the occurrence of obliterative bronchiolitis without recognized accidental over-exposure is a paradigm that contributed to recognition of deployment hazards for U.S. soldiers (King et al., 2011) and reporting of obliterative bronchiolitis cases in boat builders and others using plastic-reinforced fiberglass (Cullinan et al., 2013; Chen et al., 2013; Kreiss, 2013). The need to improve the quality of commercial spirometry for medical surveillance of excessive decline in lung function led to development of longitudinal decline freeware, retraining requirements for technicians performing occupational spirometric surveillance, and improved NIOSH-certified training courses. For an irreversible disease, early recognition before overt clinical disease and spirometric abnormalities can best prevent disability. A current challenge is identifying a mechanism for this kind of early recognition. Another challenge is developing an understanding of toxicology mechanisms and dosimetry which enable safer work practices for complex mixtures and move beyond chemical-by-chemical regulation.

The advances in toxicology of the α-dicarbonyl compounds are a compelling reminder of the danger of substitution without inhalation toxicology evaluation of potential substitutes and of the need for prudent protection from inhaled mixtures when toxicology is unavailable on constituents or mixtures of agents. These take home points are now pertinent to the constituents of e-cigarettes which often contain diacetyl-containing flavors (Allen et al., 2016). There is also renewed exploration of the role of pyrolysis-derived and flavoring-derived diacetyl from cigarettes and e-cigarettes in the small airways disease and emphysema of smokers and vapers.

7. Frontiers

Much remains to be explored in this evolving scientific area. Improved methods are being explored to measure diacetyl exposure from encapsulated (spray-dried) particulates used in flavoring. New mobile tools are now available which may enable self-monitoring of spirometry by workers until workplace exposures are controlled and quality commercial spirometry is consistently available. The recognition of pyrolysis products that include diacetyl suggests that respiratory hazards may extend to other industries. We have little insight into the dose-rate effects of diacetyl exposure, although brief high exposures from microwave popcorn popping quality control work appeared to be hazardous, despite low average exposure (Kreiss et al., 2002; Kanwal et al., 2006). Workforce epidemiology studies should assess olfactory function in relation to diacetyl exposure, as prompted by pathologic effects in rodent experiments. As mechanisms become clarified, biomarkers may require evaluation in field epidemiology studies, as potential tools for early recognition of hazardous exposures and prevention of occupational lung disease. And, within the field of toxicology, prediction of unrecognized hazards through structure activity relationships may facilitate worker health without the time and expense of inhalation studies.

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