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Diacetyl-Induced Lung Disease

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Contents

Abstract

Diacetyl is a diketone flavouring agent that is commonly employed for buttery taste as well as other purposes. Industrial exposure to flavouring agents, particularly diacetyl, has recently been associated with bronchiolitis obliterans, a severe respiratory illness producing fibrosis and obstruction of the small airways. This has been most commonly reported in the microwave popcorn production industry, but it has occurred elsewhere. In addition to bronchiolitis obliterans, spirometry abnormalities (fixed airflow obstruction) and respiratory symptoms have been associated with exposure. A direct effect on the respiratory epithelium with the disorganised fibrotic repair appears most likely as the underlying mechanism. Current data suggest that diacetyl is the agent responsible, although it is possible that diacetyl is simply a marker for another causative agent.

Bronchiolitis obliterans is a severe, often fatal, lung disorder. Work with flavouring products, particularly diacetyl, has been associated with this illness. This article will review the clinical manifestations, nature of exposures, epidemiological investigations, laboratory investigations and possible mechanisms. In addition, the article will suggest operational case definitions and

directions for future clinical practice and research. The article considers both diacetyl-induced bronchiolitis obliterans (DiBO) and the earlier manifestations of toxicity.

1. Case Reports

Several case reports focused attention on this problem. The most important case reports were from a microwave popcorn facility in Missouri. In 2000, Dr Allen Parmet recognised a cluster of cases of severe lung disease from a single plant.[1] One of the three also had significant work-related corneal irritation. He requested assistance from the state health department and subsequently from the US National Institute for Occupational Safety and Health (NIOSH), which conducted more in-depth assessment of the cluster and found eight cases had developed between 1993 and 2000.[2] Four had worked in packaging and four in the flavouring mixing area.

After these case reports were published, additional information came to light, showing there had been prior suspicion of a problem, although the link to diacetyl *per se* was not established. For example, Lockey et al.[3] reported five cases of bronchiolitis obliterans from prior experience in flavouring plants, but were unable to definitively evaluate linkage. The concerns were reported to the company involved and subsequently to the trade association in 1996.[4]

In 1983, NIOSH had investigated a plant producing flavours for the baking industry.[5] The investigation was prompted by two workers with severe obstructive disease – both worked in the mixing room. The first case, a nonsmoker, developed symptoms 7 months after beginning work; he then developed severe irreversible airflow obstruction and left employment. His replacement in the mixing room, also a nonsmoker, developed the same problem within 1 year. In addition, there were two former workers with obstructive abnormality. NIOSH did not directly prove an association with diacetyl at that time, possibly because the workers were smokers, and there were multiple exposures. Diacetyl was present, but constituted <1% of materials used in the plant.

Reports of individual cases were seen in California,[6] Maryland and North Carolina. Notably, many of these cases were from outside the popcorn production industries but were with downstream users (e.g. the California cases were in flavouring products manufacturers).

2. Case Series and Worker Surveys

The initial case reports were followed by more systematic investigations, including surveys of several plants as well as more extended case series.[7-11] Results pointed to a clear association between a particular chemical agent (diacetyl) and bronchiolitis obliterans. In addition to identifying cases of bronchiolitis obliterans, the surveys systematically administered spirometry testing and questionnaires; this added two important elements. First, less severe, presumably early, effects on symptoms and lung function could be identified. Secondly, the adequate sample size and systematic approaches allowed statistical risk factor evaluation for several outcomes: bronchiolitis obliterans, physiological airflow obstruction and respiratory symptoms.

Most of the plant surveys reported in the scientific literature were done by NIOSH. However, a trade association, Flavor and Extract Manufacturers Association (FEMA),[4] has been reported to sponsor surveys done under the direction of a faculty member at the University of Colorado. These surveys have not been released, but preliminary reports indicate less abnormality than reported by NIOSH.

The Missouri plant exemplifies the findings.[2] In the initial cross-sectional survey, 21 of 116 subjects showed airflow obstruction on spirometry testing; the prevalence was associated with quartile of cumulative exposure. Most had normal chest radiographs and normal measurements of the diffusing capacity of the lung for carbon monoxide (D_{LCO}). Several symptoms, including cough and wheeze, were increased in relation to the statistically expected frequency. Follow-up surveys were conducted for several years, showing that many had excessive rates of decline of the forced expiratory volume in 1 second (FEV_1).[10]

Subsequent case series produced generally similar results, confirming the relatively high frequency of fixed obstructive disease based on spirometry testing seen in locations with high exposures. An Ohio popcorn plant was investigated after identification of one worker with probable bronchiolitis obliterans; the subsequent survey identified fixed obstruction in 3 of 12 workers in the highest exposure areas.[11] A survey of a plant in Iowa showed that 6 of 13 mixers had obstructive disease, three of which were nonreversible (consistent with the previously observed diacetyl pattern).[12] However, there was no excess abnormality in non-mixing areas, probably because this plant had employed exposure control measures in other areas. A popcorn plant survey in Illinois also identified excess respiratory symptoms and some increase in the prevalence of spirometric abnormality.[9]

3. Diacetyl: the Agent

Diacetyl is a 4-carbon, 2-ketone chemical agent (figure 1). It is also known as 2,3-butanedione; 2,3-butadione; 2,3-diketobutane; dimethyl diketone; and dimethylglyoxal.[13] At room temperature, diacetyl is a green-yellow liquid. The low boiling point, 88°C, contributes to the hazard since it enters the vapour phase at temperatures commonly present in production processes. Its

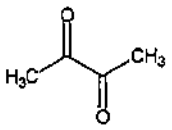

Fig. 1. Diacetyl.

vapour pressure, 178mm, also contributes to the amount present in the gas phase in workplaces. Diacetyl has good warning properties, including its obvious odour and nasal and conjunctival irritation.

3.1 Occurrence and Use of Diacetyl

Diacetyl is most widely used as a flavouring agent, providing a buttery taste. It is also used for a butterscotch-like flavour and for providing 'creaminess'.[14] It has been widely used in the popcorn production industry, but has many other applications. Some cases have been found in plants producing flavours for the baking industry.[3,5]

Diacetyl occurs naturally in many foods, such as butter, bay oils, beer, coffee, vinegar and other food products. Diacetyl occurs naturally due to microbial action and/or interactions during processing of beverages such as beer and wine; it may improve or detract from the olfactory qualities of the drink.[15,16] Distilled beverages, including whiskey and cognac, owe part of their flavour to this material.[17]

Other commercial uses for diacetyl include decreasing pathogenic bacteria in ground beef and oyster processing.[18]

The US Occupational Safety and Health Administration (OSHA) estimates that approximately 138 plants currently manufacture butter flavour popcorn, putting 3400 current employees at risk in the manufacturing process.[13] However, it is likely that the number of workers in other settings is much greater. The actual number of food processing end-users is unknown, but the wide use of the agent suggests many possible locations with exposure. FEMA estimates that about 96 000kg of diacetyl were used in 1995.

4. Bronchiolitis Obliterans: Overview

This section will briefly review characteristics of bronchiolitis obliterans in general.

4.1 Histopathology

Bronchiolitis obliterans is a relatively uncommon pulmonary disease that is characterised by airway inflammation and fibrosis at the bronchiolar level.[19] The disorder shows proliferative changes of the airway, leading to luminal obstruction. The scarring may be eccentric, leading to partial obstruction of the small airway, or may completely obstruct the lumen. Fibroblasts and collagen deposition are present. In addition to fibrotic changes, inflammatory findings are noted. As discussed in section 7, the nature of the inflammatory infiltrates in DiBO differs from that of the more frequent cases of bronchiolitis obliterans. The pathological findings are generally specific for the disorder. There is considerable inhomogeneity from area to area within the lungs. In addition, there often are areas of bronchiectasis, showing irregularity and dilatation of the bronchioles.

4.2 Physiology

Bronchiolitis obliterans produces two main physiological effects: (i) obstruction to airflow; and (ii) air trapping/hyperinflation. The obstructed airways prevent rapid emptying of more distal lung units during expiration. The involved areas of the lung show air trapping, because air cannot exit adequately from the affected units due to partial obstruction of the airway. This leads to an overall increase in the volumes of the lungs, termed hyperinflation. Hyperinflated lungs have increased elastic recoil due to the stretch of elastic tissue; this may partially compensate for the obstruction to exhalation.

Pulmonary function testing reveals a specific pattern in classical cases: there is severe obstruction to airflow, manifested by a greatly reduced FEV_1 and ratio of the FEV_1 to the forced vital capacity (FVC) [reduced FEV_1/FVC ratio]. However, the airflow obstruction is not reversible by use of inhaled bronchodilators. Several physiological features distinguish bronchiolitis obliterans from more common causes of the severe airflow obstruction such as asthma and chronic obstructive pulmonary disease (COPD). Asthma shows reversibility – the degree of airflow obstruction (FEV_1/FVC ratio) varies considerably from day-to-day and the FEV_1 is generally improved after administration of a short-acting bronchodilator; this response is not seen in bronchiolitis obliterans. COPD, such as emphysema, is also characterised by severe airflow obstruction; however, it is almost always accompanied by reduction in the diffusing capacity of the lung for carbon dioxide when the airflow obstruction is severe. Furthermore, many cases of COPD have some degree of airway hyperresponsiveness. Bronchiolitis obliterans may be distinguished from pulmonary fibrotic disorders (e.g. usual interstitial pneumonitis/idiopathic pulmonary fibrosis or asbestosis) by the presence of airflow limitation as the primary manifestation rather than reduction in the FVC on spirometry. Furthermore, interstitial pulmonary fibrotic disorders are characterised by a reduction in the total lung capacity (TLC), whereas bronchiolitis obliterans has increased TLC.

Bronchiolitis obliterans affects predominately the small airways. Therefore, some have suggested that the flow rate of the

midportion of the expiratory spirogram (the $FEF_{25-75\%}$) might be useful as a screening tool for detecting early bronchiolitis obliterans.

In addition to spirometry, measurement of the diffusing capacity of the lung for carbon monoxide (D_{LCO}) and measurement of lung volumes are diagnostically useful. The former is typically much less abnormal than the spirometry in bronchiolitis obliterans. Conversely, lung volume testing shows lung volumes that are much larger than normal, reflecting the hyperinflation and air trapping.

4.3 Radiographic Imaging

Radiography of bronchiolitis obliterans shows patchy areas of fibrosis and hyperinflation. While the standard chest radiograph may be nonspecific, the CT scan typically shows scattered areas of fibrosis and hyperinflation (lucency). The pattern is described as 'mosaic', meaning intermixing of affected and unaffected areas.

Radiographic findings can help distinguish bronchiolitis obliterans from other respiratory disorders. In COPD, areas of emphysema (diffuse hyperinflation/ lucency, bullae and other manifestations) are initially seen mainly in the upper lobes and are less patchy. Asthma is generally without manifestations other than hyperinflation. The distribution of bronchiolitis obliterans findings is patchy, leading to the mosaic findings on radiographic testing. This differs from the typical pattern seen in comparably advanced cases of pulmonary fibrosis or emphysema, which are more uniform and diffuse.

CT scans also may show the areas of deformation and dilatation of airways consistent with bronchiectasis.[20]

4.4 Symptoms and Physical Examination Findings

Patients with bronchiolitis obliterans generally develop progressive shortness of breath, often accompanied by cough without sputum production. Because of the insidious nature of the progressive symptoms, they may not notice the onset. Examination in the early phases may be normal or show limited findings of hyperinflation. Later in the disease process, end inspiratory crackles and wheezes may be observed.[21] Thus, medical examinations for symptoms and physical examination are neither sensitive for early detection nor specific for the illness.

4.5 Causes of Bronchiolitis Obliterans

Flavouring product exposure is still a relatively uncommon cause of bronchiolitis obliterans. By far, the most common aetiology is rejection following lung transplantation. Other reported causes include connective tissue disorders, especially rheumatoid arthritis; viruses, such as respiratory syncytial virus; other infections such as mycoplasma pneumoniae; drugs such as d-penicillamine; radiation; ulcerative colitis; and ingestion of the East Asian vegetable *Sauropus androgynous*; and inhalation of cocaine. Industrial exposures causing bronchiolitis obliterans include nitrogen oxides, chlorine gas and other chlorides (such as zinc chloride, phosgene), ozone, hydrogen sulphide and sulphur dioxide.[19]

4.6 Other Diagnostic Methods

Bronchoalveolar lavage is a bronchoscopy procedure by which cells within the lung airspaces (distal airways and alveolar regions) are obtained by wedging a bronchoscope in an airway and then instilling liquid to wash out cells. The lavage fluid is analysed for cell types and proportions. Inflammatory cells such as granulocytes, lymphocytes and eosinophils may be counted. In addition, various mediators such as interleukins (IL) may be assayed to provide mechanistic insight.

In lung transplant patients, bronchiolitis obliterans is characterised by increased granulocyte and lymphocyte counts in bronchoalveolar lavage fluid, as well as eosinophils granulocytes and higher concentrations of IL-6 and IL-8.[19]

Exhaled nitric oxide has also been studied as a marker of early effects such as bronchiolitis obliterans. In lung transplant patients, an increase in exhaled nitric oxide, presumably from epithelial inducible nitric oxide synthase, precedes bronchiolitis obliterans in this group.[21]

5. Human Studies

Pathology studies were done on several biopsy specimens from patients with DiBO.[2,6] Unlike post-lung transplant bronchiolitis obliterans, DiBO cases do not show lymphocytic infiltrates. Bronchiolar inflammation and fibrosis were seen. Notably, non-caseating granulomas were reported in two cases.[2,6]

One study obtained induced sputa samples from exposed workers, including 59 with 'high' and 22 with 'low' exposures.[22] No unexposed controls participated, but results were compared with data from a published study of normal research volunteer subjects. The exposed non-smoking workers had higher neutrophil concentrations than normal subjects. In addition, there was a correlation of neutrophil concentration with exposure status – concentrations were greater in the high- than the low-exposed group. Two other inflammatory markers (IL-8 and eosinophil cationic protein) showed comparable patterns. However, although not reaching statistical significance, a trend towards an association between neutrophil concentration and rate of decline of the FEV_1 over time was observed.

Radiographic studies of DiBO cases generally show characteristics similar to those reported for transplant-related bronchiolitis obliterans.[23]

6. Animal Studies

Several studies provide insight into the mechanism of injury. Overall, they suggest a direct effect upon the airway epithelium.

The earliest known inhalation animal study of diacetyl was completed by a manufacturer, BASF.[24] Three groups of ten Wistar rats were exposed for 4 hours of diacetyl inhalation at three concentrations (2.2, 5.2 and 23.9 mg/L) to determine the concentration in air that is lethal to 50% of the animals tested (i.e. the LC_{50}). All rats died at the two higher concentrations. Thus, the estimated LC_{50} for both male and female rats was $2.25 < LC_{50} < 5.2$ mg/L. Rats in all three groups were described as having 'dragging respiration'.

Both morphological and physiological effects have been demonstrated in experimental laboratory studies. Inhalation studies have demonstrated morphological changes in the respiratory tract due to short-term (6-hour) exposure to vapours from heating butter flavourings. In a rat model, three concentrations of diacetyl (203, 285, 352 parts per million [ppm]) as part of the complex butter flavouring vaporisation were studied.[25] In addition to the 6-hour continuous exposure, 'pulsed' methods were used to simulate workers with intermittent high exposures as during intermittent pouring and mixing tasks; the average concentrations were similar to the continuous exposures (e.g. the high pulsed average was 371 ppm, but the instantaneous levels varied from 72 to 940 ppm). Necrotic denudation of the airway epithelium was seen at several levels, including nose and trachea. Effects were greater in the large airways, but were also noted in the smaller airways. Inflammatory responses were granulocytic both in tissue and in bronchoalveolar fluid. The extent of damage was greater at high exposure compared with lower concentrations. Nasal effects were seen even at the lowest exposure levels, suggesting greater sensitivity of the nose. The experimental exposure studies also examined the alveolar region and demonstrated that this is not comparably affected.

Isolated perfused airway explants have been employed to demonstrate physiological impact.[26] In such preparations, diacetyl produced increased responsiveness to methacholine. In these experiments, isolated perfused trachea was used. Methacholine, an acetylcholine analogue, was used as a measure of airway responsiveness. When applied to the mucosal side of the tissue explants, significantly increased responsiveness was noted; however, when the methacholine was applied to the serosal (outside) of the organ model, only minimal increases in responsiveness were noted. The receptors for methacholine are typically located just beneath the mucosal epithelial surface. Thus, the finding of markedly increased responsiveness to mucosal but not to serosal application suggests that the diacetyl injured the mucosal barrier, which normally protects the sub-epithelial irritant receptors. This model was employed to measure the electrical potential, here also showing that there was a loss of electrical potential gradient. The latter studies also show dose responsiveness and relative specificity for diacetyl, since the diacetyl alone demonstrated significant responses.

The studies show that the mechanism is a direct toxic effect leading to airway epithelial inflammation and loss of integrity. The physiological studies are consistent with the morphological studies, showing the airway epithelium as the primary tissue target.

The cellular response was nearly completely neutrophilic; the studies did not demonstrate lymphocytic responsiveness, granuloma formation, or other indicators of immunological responses. The mechanism is unlikely to be immunological in nature.[1]

There are differences between the experimental responses and those seen in humans. The disease in humans is characterised by a fibrotic, airway constrictive and proliferative response. However, the experimental models show only the acute response. The basis for transition from neutrophilic inflammation to a fibrotic response focus in particular airway segments is not yet understood.

7. Mechanism of Toxicity

Lung transplantation is frequently followed by bronchiolitis obliterans as the main manifestation of rejection. Post-lung transplant bronchiolitis obliterans has received considerable attention since this is a leading cause of post-surgical death.[19] The early rejection mechanism is likely to differ from DiBO. While lymphocytic infiltration is seen after transplantation, the effect of diacetyl is mainly granulocytic.

It appears likely that diacetyl causes direct injury to the airway epithelium. The consequent repair process is disorganised, producing uncontrolled fibroblastic and myoblastic proliferation.

The mechanism of DiBO is not known. As discussed in section 4.1, the pulmonary response, bronchiolitis obliterans, differs from the more common inflammatory patterns (e.g. asthma, diffuse pneumonitis or alveolar inflammation). The fundamental mechanism is unlikely to include an allergic sensitisation mechanism. Specifically, the early-onset argues against mediations by specific antibody induction; furthermore, such effects typically affect larg-

1 The predominance of neutrophilic (rather than lymphocytic) response in laboratory animals is consistent with the lack of response to corticosteroid therapy in humans.

er airways leading to airway hyper-responsiveness (e.g. occupational asthma), diffuse interstitial fibrosis, or granulomas. Granulomas are seen occasionally in DiBO, but are not a routine manifestation, also arguing against a hypersensitivity pneumonitis type of response as the underlying aetiology.

To date, studies have not shown whether less severe insult to the airway epithelium may be followed by full repopulation of the airway. The extensive damage in the very high dose inhalation studies leaves little regenerative capacity.

As of today, mechanistic studies leave much uncertain. While the studies demonstrate airway neutrophilic inflammatory response, they do not define the particular target cell *per se*. Furthermore, results did not describe the mechanism of toxicity, nor do they elucidate any upstream events affecting transcriptional control. They also do not identify a specific metabolic effect. Studies to date have not elucidated the actual components of the inflammatory cascade. Such mechanistic information may be critically important to determine appropriate therapeutic interventions.

Studies have relied upon acute exposures in a moderate to high level. The effect of repetitive low-dose exposure has not been assessed in animal studies.

Diacetyl is water soluble. Animal studies have not shown if this solubility is sufficient to protect the lower (intrapulmonary) airways from low concentration inhalation by dissolving the diacetyl in the fluid lining the nose and pharynx. Water solubility in the upper airway may account for the greater effect on the nose seen in the rat inhalation study.[25]

The underlying biochemical interaction between diacetyl and tissue *in vivo* is unknown. *In vitro* chemistry studies have shown that high concentrations of diacetyl react with the amino acid arginine. The reaction is reversible except in the presence of borate.[27] Chemical research into the cause of malodorous wine found that diacetyl interacts with cysteine.[16] The relevance of these findings to human disease is uncertain.

8. Diacetyl versus Flavourings as the Cause

There are compelling data associating flavouring exposures, particularly high exposures, to the development of bronchiolitis obliterans. There are three possible relationships between diacetyl and bronchiolitis obliterans: (i) diacetyl is the sole cause; (ii) diacetyl is one of many different flavouring plant agents causing the disease; (iii) diacetyl is simply a marker of exposure, and another, yet unidentified, agent(s) is the proximate cause. The available data do not completely resolve the question of, "is diacetyl the real cause"?

Findings suggesting that diacetyl is the primary and possibly the only cause include the following:

- The case reports all involved plants and workers with particularly extensive exposure to this specific agent. Because many of the cases were discovered anecdotally rather than based on systematic investigations of the plants, such association is unlikely to be due to ascertainment bias. For example, the first two cases had very extensive exposure to this specific agent, yet neither was referred specifically because of diacetyl exposure.
- Epidemiological data support association between diacetyl work *per se* and elevated risk. For example, several studies have shown a statistical relationship between exposure and the risk of both fixed obstructive abnormality and of bronchiolitis obliterans *per se*.
- Animal studies have demonstrated that experimental exposure to diacetyl alone can produce significant inflammation localised to the airway.

Conversely, some feel that the association of diacetyl with bronchiolitis obliterans is nonspecific. They note that the experimentally induced lesions in the animal models are not bronchiolitis obliterans *per se*, but are limited to inflammatory responses. There are numerous exposures in the flavourings industry, and few, if any, of the cases have been exposed only to diacetyl. Flavouring agents used much less frequently or in lower quantities may be the true causative agent(s), particularly if an immunological mechanism was involved. Because diacetyl is used in higher concentration, it may be incorrectly identified as the causative agent. That is, it is theoretically possible that diacetyl may simply be a convenient 'marker of exposure' rather than the causative agent *per se*.

While the matter is not fully resolved, the data in aggregate support the conclusion that diacetyl appears likely to be particularly hazardous and must be subject to control. However, it is critically important to ascertain if other agents also constitute a risk and warrant similar stringent controls.

9. Dose Relationship

The data support a dose-response relationship between diacetyl and both bronchiolitis obliterans and fixed airflow obstruction. As shown in table I, the diagnosis of bronchiolitis obliterans was concentrated in workers with heavy exposure. The case reports initiating concern were for workers with very heavy exposures (e.g. mixing). Worker surveys and epidemiological studies have shown a relationship between exposure and the proportion of workers affected, as well as between exposure and the magnitude of effect. For example, Kreiss et al.[2] demonstrated a relationship between quartile of cumulative exposure and proportion of workers with airflow obstruction. Thus, there are both dose-response

Table I. Suggested case definitions and criteria

Purpose	Exposure history	Exposure quest.[a]	Respiratory symptom quest.[a]	Spirometry	Post-BD spirometry	Full PFT[b]	Chest x-ray	CT scan	Lung biopsy[c]	BAL	Plant IH survey	Serology[d]
Clinical diagnosis and treatment	M		M	M	M	M	M	M	A			M
Screening for early signs (secondary prevention – case detection)		M	A	M	M						A	
Population surveillance			A	M								
Hazard surveillance (indirect exposure assessment)		M									M	
Epidemiological study		M		M	A						M	
Case registry												
specific for DiBO		M	M	M	M			M				
as part of an existing lung disease surveillance system (e.g. SENSOR)		M	M	M	M			M				
Research into mechanisms		A	A	A	A	A	A	A	A	A	A	A
Compensation												
tort litigation	M								A		A	M
workers' compensation	M			M	M	M	M	M				A

a Standardised questionnaire.

b Spirometry and post-BD spirometry, and diffusing capacity and lung volume.

c Video-assisted thoracoscopy or open biopsy.

d Blood tests for collagen vascular disorders.

A = advisable; **BAL** = broncholalveolar lavage; **BD** = bronchodilator; **DiBO** = diacetyl-induced bronchiolitis obliterans; **IH** = industrial hygiene; **M** = mandatory; **PFT** = pulmonary function test; **quest.** = questionnaire; **SENSOR** = Sentinel Event Notification System for Occupational Risks.

and dose-effect relationships. Milder manifestations and animal models also support dose responsiveness.

The contributions of cumulative dose versus short-term peak exposure are uncertain. Short-term animal exposures indicate that concentration alone may determine the magnitude of the early inflammatory response, while human data are indeterminate since both cumulative exposure (i.e. work years) and magnitude of exposure (i.e. job title, task) are correlated. No human cases have been reported after only a single exposure. It is unknown whether a threshold exists. Both the early (LC_{50}) and recent animal studies employed high concentrations.

There is increasing concern that similar effects might occur at lower levels. Is there a minimum threshold level warranting concern?

There are no thorough studies of end-users of diacetyl-containing foods. Although quality control workers in popcorn facilities may be exposed to levels comparable with those in flavouring production facilities, most other end-users are likely to have considerably lower exposure. Nevertheless, because so many more persons are potentially exposed, there is still significant concern. Therefore, the US Environmental Protection Agency has initiated an exposure analysis relevant to nonoccupational settings, but results are not likely to be available in the immediate future.

10. Case Definition

A completely definitive diagnosis of DiBO requires a lung biopsy, lung function testing, careful medical evaluation to exclude other causes of the disease (e.g. collagen vascular diseases) and specific worksite assessment to document the exposure. Needless to say, such assessment is generally not feasible. Therefore, operational case definitions are required for specific purposes. These purposes are listed in table I.

A clinical definition is used to provide the basis for a specific intervention, whether diagnostic or therapeutic. A greater degree of certainty is needed before submitting a patient to a potentially hazardous diagnostic manoeuvre (e.g. lung biopsy) or therapy than for collecting cases in a surveillance study. Therefore, diagnostic criteria for the clinical purposes must be more specific than for epidemiological purposes. Similarly, the degree of certainty for compensation purposes must depend upon the philosophy surrounding the situation, such that workers compensation typically requires less diagnostic certainty than tort suits.

Screening programmes should be sensitive even at the cost of some specificity. For example, a screening programme should be implemented in order to delineate individuals who warrant more in-depth (and expensive) follow-up testing. Similarly, screening may find individuals for whom the 'precautionary principle' warrants removal from exposure even if it is not highly probable that they have DiBO. Several diacetyl-related studies have data suggesting that removal from exposure may reduce progression of abnormality. For example, those hired after implementing control measures in the index plant had less abnormality,[2] and several early cases had no worsening after leaving employment.[3,23]

Public health surveillance applications generally require a highly sensitive approach, not necessarily as specific as for clinical purposes. For epidemiological research purposes, relatively easy to administer testing procedures such as questionnaires or spirometry are used. Conversely, for epidemiology, the testing must be more standardised than for clinical diagnosis, in which 'good clinical judgment' may be involved.

Case registries generally rely upon reporting of individual cases by community-based clinicians. They may serve multiple purposes. First, a registry permits reporting of the aggregate characteristics of those reported to have the disease. Secondly, a registry may provide some information about the frequency of a disease. Case registries, however, are limited to the cases that are voluntarily reported. Thus, they may not be completely representative. Furthermore, the operational definition of a 'case' and the type of clinician awareness programmes very much affect the data acquired. A registry approach cannot actually determine the incidence of a disease because it only has 'numerator' data (number of cases) but does not delineate the number of persons at risk (i.e. the 'denominator'). It is also sensitive to the effectiveness of case capture (what percentage of existing cases are actually reported to the registry?). Some case information may be acquired from existing general lung disease surveillance systems, such as the SENSOR (Sentinel Event Notification System for Occupational Risks) programmes operated by several health departments and NIOSH. Conversely, a case registry may be specifically developed for DiBO. NIOSH has instituted a disease-specific case registry; information concerning this is available online.[28]

In actuality, there are two distinct but interrelated disorders: DiBO *per se* and fixed obstructive spirometric abnormality. Meeting the criteria for the latter should not imply that the former is present.

A single-case definition is unlikely to fulfill all of these needs. Therefore, we propose the operational definitions summarised in table I. The extent of testing differs according to the purpose.

11. Exposures

Studies of production facilities found a large number of volatile organic compounds (VOCs). For example, >100 compounds were present in air samples.[29] In-laboratory testing of butter flavour

samples also demonstrated the complexity of the mixtures.[25] However, diacetyl *per se* is the major constituent.

Airborne exposure is the primary route of concern. Because diacetyl is a mucous membrane irritant and has a distinctive odour, it can be detected easily. The most significant effects are due to inhalation. Eye and mucus membrane irritation as well as the more serious respiratory effects are related to inhalation exposure. The liquid state has a significant vapour pressure, leading to evaporation of liquid. In addition, diacetyl has a relatively low boiling point, such that temperatures present in processing are sufficient to produce high air concentrations. Furthermore, aerosol exposures occur from the bulk transfer operations, creating liquid aerosols or dust aerosol depend upon the physical state.

Several factors determine the extent of exposure:

1. Total bulk quantity used. The case reports have all come from locations with very extensive use.

2. Physical state of diacetyl. Diacetyl may be used as either a liquid, paste, or as a solid powder. Laboratory testing of 40 butter flavourings has shown that the liquid and paste forms lead to much higher air concentrations than the powder forms.[30] Boylstein et al.[30] found that the average air concentration with liquids and paste was nearly always higher than with powder forms. The solid forms are produced by affixing the liquid flavouring agent to a solid substrate. Two methods are used: in plated flavours, the diacetyl may evaporate before use, whereas the encapsulated spray dry types limit volatilisation before actually popping in a microwave oven.[30] While the solid forms lead to lower air levels, caution is needed. The particle size is respirable, possibly serving as a vehicle to carry the active agent to the bronchioles. The solid aerosol form may reduce the protective effect of dissolution of vapours in the water lining the upper respiratory tract.

3. Work operation. The specific work operation greatly affects the air concentrations. The workplace surveys demonstrated that air levels are particularly high in mixing operations.[29] Packaging operations are also associated with high levels. The mean air concentration of diacetyl was 38 ppm in mixing tank rooms and 2.0 ppm in microwave-packaging area, but only 0.02 ppm in other areas of the plant.[29] The quality control work area also had high levels since each employee popped at least 100 bags per shift in a poorly ventilated room. The average concentration was 0.54 ppm, with as much as 13 ppm when opening a recently popped bag. In addition to direct measurement, the case reports and worker surveys show that both fixed airway obstruction and bronchiolitis obliterans are clustered within the high-exposure operations.

4. Task. Several specific tasks are associated with high peak concentrations. Most notably, pouring both diacetyl into open vessels creates high air levels in the immediate proximity. The risk of mixing operations is likely to be much greater when the vessel into which the flavouring is poured is heated.

5. Work performance characteristics. For example, headspace concentrations directly above a heated mixing vessel were much higher than elsewhere, so a worker who physically leans over a vessel is likely to get high peak exposures. Unexplained headspace concentration differences from day to day (e.g. 383–1230 ppm) may be due to undetected differences in local exhaust ventilation performance.[10] The speed with which flavouring agents are poured also determines air levels; for example, in a simulated work task, hurriedly dropping material into a tank increased emissions markedly compared with slower transfers.[30]

6. Exposure controls. As discussed in section 11.1, workplace exposure controls can significantly reduce exposures.

11.1 Exposure Control

Exposures have been studied in several microwave popcorn plants described in technical reports from NIOSH. Industrial hygiene studies conducted before and subsequent to implementation of recommended exposure controls demonstrated the efficacy of the methods in reducing exposure significantly. For example, between 2001 and 2003, exposure controls were instituted in the Missouri plant. In the mixing room, average diacetyl concentrations fell from 38 ppm in 2001 to 0.46 ppm in 2003; in packaging, the average concentration was reduced from 1.69 to 0.002 ppm.[10] Another report indicated reduction in peak exposures during mixing from 462 to 0.97 mg/m^3.[31]

Exposure controls have included improved exhaust ventilation. In addition, isolation of the mixing area protected other areas of the plants. Ultimately, use of closed processes rather than batch process methods has been recommended.

In addition to ventilation and isolation, respiratory protection of workers has been strongly recommended. Powered air purified respirators, which are air-purified respirators utilising a fan for airflow rather than relying upon workers' inhalation, were suggested. Such devices provide a higher level of protection since worker inhalation no longer creates as negative a pressure inside the mask as with typical air purifying respirators; negative mask pressure allows external contaminants to enter. Powered air purified respirators provide considerably greater protection than conventional purifying respirators, but are not as protective as atmosphere supplying devices. Nevertheless, they are much more practical.

Exposure controls should address both cumulative (average) and peak exposures. The data are currently inadequate to determine if short peak excursions alone, in the presence of low average exposures, produce disease. Kanwal et al.[32] reviewed data from

several plants and concluded that peak exposures may constitute a risk since disease developed even where average exposures were only 0.02 ppm.

12. Health Surveillance

Workers potentially exposed to diacetyl or other suspect flavouring agents should be periodically medically evaluated. Although not absolutely proven, the available data support the potential benefit of screening for both disorders: bronchiolitis obliterans and fixed airflow obstruction.

Identifying bronchiolitis obliterans, even if fully manifest at the time of diagnosis, may benefit the individual. Although treatment to reverse the damage is not available (except lung transplantation), removal from further exposure may prevent progressive damage.[3,22,23] Importantly, finding such cases may lead to programmes to protect other current and future workers. Because of the short latency for developing bronchiolitis obliterans, a sentinel case may encourage exposure controls to protect others. This contrasts with the situation for asbestos exposure, which typically takes 20–30 years from first exposure until manifesting disease clinically; for asbestos, by the time a case is detected, work conditions are likely to have changed already. Furthermore, because bronchiolitis obliterans is an unusual disorder, even a single otherwise unexplained case should warrant concern.

Spirometry testing is commonly employed in occupational settings with respiratory risks. It is particularly helpful for screening workers with possible diacetyl exposure because it is well standardised and facile to administer. The pattern of fixed obstructive abnormality (reduced FEV_1/FVC ratio without bronchodilator response) can help distinguish this from other more common respiratory disorders, particularly in young workers and non-smokers. Individuals with abnormal results should be evaluated in more detail, including careful assessment of other risk factors and conducting more complete lung function testing. While not proven, it is reasonable to assume that fixed airflow obstruction is an early stage along the path from normality to clinically severe bronchiolitis obliterans.

In view of rapid progression of the disorder, in which some cases have developed with only a year of exposure, discretion is needed. Spirometry testing on an annual frequency will not detect all cases developing in the inter-test interval. Therefore, medical screening cannot be considered as a substitute for environmental control.

In the future, complementary information may be obtained from the use of sputum markers of inflammation[22] or measurement of exhaled NO. However, these techniques are not currently generally available, nor do they (yet) have adequately demonstrated value in flavouring-exposed workers to warrant general use.

13. Regulatory Standards

The FEMA trade association has been sponsoring an Expert Panel to regularly review the safety of flavouring agents for 40 years, beginning soon after 1958 regulatory actions of the US FDA.[33] The FDA created a list of materials considered to be generally recognised as safe (GRAS). The FEMA Expert Panel periodically reviews flavouring products to determine if they should be considered GRAS. The review focuses on use "...under conditions of intended use in food." The Panel considers toxicity in relation to the potential intake in food[33] (i.e. they do not explicitly consider workplace exposures *per se*).

There currently is no OSHA permissible exposure limit (PEL) or NIOSH recommended exposure limit (REL) for diacetyl. Recently, a petition has been filed by two unions to urge the establishment of regulatory standards in the US.[34] There are PELs or RELs for only 46 of the 1037 flavouring agents identified by the industry's expert panel as potentially affecting the respiratory system.[4,9]

Referring to diacetyl and acetaldehyde, FEMA[4] concluded in 2004 that "...neither substance has been shown to be a cause of this respiratory illness...". FEMA further noted that "diacetyl... is cited by NIOSH as a marker of exposure in microwave popcorn manufacturing and as a substance that can cause airway injury in animals". The association did, however, caution its members to consider diacetyl a 'high priority' substance and recommended exposure controls.

DiBO illustrates the strengths and limitations of the information diffusion process. In retrospect, there was evidence of serious respiratory disease in flavouring work at least as early as 1985 and 1995.[3-5] Investigation did not occur because there is no easy mechanism to facilitate such investigations, particularly with a private employer. A serendipitous event led to focusing attention on 'microwave popcorn lung'. Severe respiratory disease was noted in a Missouri plant; the local health officer was particularly primed to detect and respond to workplace hazards since he had formerly been the corporate medical director responsible for a large worker population and because he was actively involved in an aerospace incident investigation (personal communication).[1] Similarly, the first two Californian cases were serendipitously referred to a university occupational lung disease specialist who is also actively involved in public health activities.[6]

14. Unanswered Questions

Major unresolved questions requiring research include the following:

- Does diacetyl alone produce bronchiolitis obliterans-like findings in an animal model?
- Does any other flavouring agent present in the facilities produce comparable effects in animal models?
- Is there a threshold for adverse effect of diacetyl (i.e. a 'no observed adverse effect level') in animals or humans?
- What are the effects of long-term low-level exposure or long-term intermittent moderate-level exposure in animal models?
- What is the biochemical interaction between diacetyl and endothelial cells?
- Can the respiratory endothelium regenerate after exposures slightly above the lowest observed effect level?
- What are early markers of inflammation in exposed humans (e.g. in bronchoalveolar lavage fluid, exhaled NO, nasal lavage markers)?
- What is the optimal frequency of and definition of 'abnormal' for spirometry screening?
- Is there a practical role for longitudinal spirometry testing (i.e. measuring change over time rather than assessing normality of each test)?
- What is the frequency and level of diacetyl exposure to downstream users, such as in baking, candy and other food processing industries?
- Do measurable significant exposures occur in consumer applications, such as home use of microwave popcorn?
- Which workplace exposure control methods are most effective and feasible?
- What are regulatory policy options?
- How can information about risk and control measures be most effectively disseminated?

15. Conclusions

Exposure to flavouring agents, particularly diacetyl, has been associated with several health outcomes: (i) bronchiolitis obliterans, a severe and potentially fatal disorder of the small airways; (ii) fixed/nonreversible airflow obstruction; (iii) respiratory symptoms; and (iv) mucosal and conjunctival irritation. Illness can develop within 1 year of starting exposure. The mechanism is likely to involve direct toxic effect upon the respiratory endothelium. While reported mainly from the microwave popcorn industry, it is clear that workers in other industries are also at risk. The at-risk industries and number of potentially affected workers is unknown. Workplace exposure control methods have already been demonstrated to be both practical and effective at reducing risk. Occupational health surveillance should include both spirometry and respiratory questionnaires. There are many unanswered questions, ranging from understanding the mechanism to public policy questions.

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