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Diacetyl in Foods: A Review of Safety and Sensory Characteristics

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Abstract: Diacetyl, noted for its appealing butter-like aroma, is present naturally in many foods, and humans have been exposed to it since the beginning of civilization. The advent of microwave (MW) cooking technology has led to the development of a significant market for MW popcorn, to which diacetyl and other flavoring compounds have been frequently added. Based upon reported associations between diacetyl inhalation and lung disease in employees of MW popcorn processing facilities, a very conservative safe level of occupational exposure to diacetyl has been proposed by the American Conference of Governmental Industrial Hygienists. Yet there is conflicting evidence that diacetyl causes lung disease in workers, and no evidence to condemn diacetyl as the cause of lung problems in MW popcorn consumers. Consumer dietary exposure to diacetyl in foods is below levels of health concern while common airborne levels of diacetyl from MW popcorn are far below the conservatively established limit to protect workers.

Keywords: aroma, diacetyl, health, popcorn, toxicology

Definitions of Select Abbreviations

ACGIH (American Conference of Governmental Industrial Hygienists)—a professional association of industrial hygienists and practitioners of related professions, which advances worker protection by providing scientific information to occupational and environmental health professionals.

FEV_1 (forced expiratory volume in 1 s)—a measure of the volume exhaled during the first second of a forced expiratory exercise started from the level of total lung capacity.

GRAS (generally recognized as safe)—a U.S. Food and Drug Administration classification for any food substance that is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use.

LOAEL (lowest observed adverse effect level)—the lowest exposure level at which there are statistically or biologically significant increases in the frequency or severity of adverse effects.

NOAEL (no observed adverse effect level)—the highest exposure level at which there are no adverse effects observed in the exposed population when compared with its appropriate control. Some effects may be produced at this level, but they are not considered to be adverse or precursors to adverse effects.

NIOSH (National Institute for Occupational Safety and Health)—U.S. federal agency, part of the Centers for Disease Control and Prevention within the Department of Health and Human Services, responsible for conducting research and making recommendations for the prevention of work-related injury and illness.

OEL (occupational exposure limit)—upper limit on the acceptable concentration of a hazardous substance in workplace air for a particular material or class of materials.

TLV (threshold limit value)—the airborne concentration of a substance below which all workers are believed to be protected while exposed to it day after day for 8-h periods.

TLV-TWA (threshold limit value-time-weighted average)—a time-weighted average concentration for a normal 8-h workday or 40-h workweek.

TLV-STEL (threshold limit value-short-term exposure limit)—maximum concentration for a continuous 15-min exposure period, for 4 such periods per day, with at least one 60-min break, provided that the TLV-TWA is met.

TWA (time weighted average)—a worker's daily exposure to a substance, or agent, averaged to an 8-h workday, taking into account the average levels of the substance or agent and the time spent in the area.

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Introduction

Food aroma is an important driver in food acceptability. Diacetyl, noted for its appealing butter-like aroma, is a naturally occurring component in many foods and is also added to a variety of foods, including microwave (MW) popcorn. The U.S. Food

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and Drug Administration (FDA) considers diacetyl to have GRAS (generally recognized as safe) status based upon centuries of human exposure to diacetyl in fermented foods, with no apparent health concerns (Birkenhauer and Oliver 2003).

In the early 2000s, clusters of obstructive pulmonary disease cases arose among long-term employees of MW popcorn production facilities in Illinois (Sahakian and others 2003), Ohio (Kanwal and Kullman 2004), Missouri (Kanwal and others 2006b), and Montana (Kullman and Sahakian 2007). An association was identified between the production of butter-flavored MW popcorn, predominantly the flavor compound diacetyl, and bronchiolitis obliterans (BO), a rare, irreversible lung disease (Kreiss and others 2002; Kullman and others 2005; Boylstein and others 2006; Kreiss 2007; Sahakian and others 2008; Lockey and others 2009; Anderson and others 2013; Zaccone and others 2013). The term "popcorn workers' lung" was popularized after these findings. More recently, 4 employees of a cookie dough production factory in Brazil developed BO as a consequence of 1 to 3 y of exposure to artificial butter flavoring vapors, specifically diacetyl, without personal protective equipment (PPE) (Cavalcanti and others 2012). Since these early reports, diacetyl has been substituted with alternative flavoring compounds; improved personal ventilation and PPE have been developed; employees have received better training; occupational exposure limits have been proposed; and follow-up site visits have been conducted (Parmet and von Essen 2002; Sahakian and others 2003; Kanwal and Kullman 2004; Boylstein and others 2006; Kreiss and others 2012; Anderson and others 2013; Halldin and others 2013; Cummings and others 2014; Hirst and others 2014; Kreiss 2014).

While the primary concerns regarding potential health risks from diacetyl and other flavoring compounds focused upon occupational exposure, Egilman and others (2011) and Egilman and Schilling (2012) warned that inhalation of diacetyl-containing flavorings may pose a significant respiratory risk to consumers of MW popcorn. In fact, in 2012, a jury awarded damages to a Colorado man who claimed that he developed "popcorn lung" from eating about 2 bags of MW popcorn every day for 10 y (Jaslow 2012).

Occupational exposure to diacetyl has been the subject of several recent reviews and is also summarized in this review. Additionally, this review discusses key food-related issues including sensory findings concerning diacetyl's contribution to food aroma and acceptance as well as the potential health risks to consumers from ingestion and inhalation of diacetyl from popcorn and other foods.

Diacetyl in Foods

Diacetyl, or 2,3-butanedione (a polar, hydrophobic diketone with the relatively simple structure of CH_3–CO–CO–CH_3), is a small, very volatile aromatic compound with a vapor pressure of 56.8 mm Hg at 25 degrees C (National Institutes of Health 2015). Commonly associated with dairy products, diacetyl is an important aroma compound in butter, margarine, sour cream, yogurt, and a number of cheeses, including Cheddar, Gouda, Camembert, Swiss, Maasdam, quarg, Mexican Chihuahua, ricotta, cottage, and goat cheeses (Dacremont and Vickers 1994; McSweeney and Sousa 2000; Smit and others 2005; Van Hekken and others 2006; Krause and others 2007; Le Bars and Yvon 2007; Attai 2009; Cheng 2010; Cruz and others 2012). Many laboratories evaluate dairy product quality based upon diacetyl content (McSweeney 2004; Le Bars and Yvon 2007; Milesi and others 2010). Antinone and others (1994) determined that diacetyl could be detected at as low as 0.2 ppm in cottage cheese and reported that consumers wanted a higher diacetyl concentration in low-fat (4 ppm) cottage cheese, compared with full-fat (1 ppm) cottage cheese. More recently, Drake and others (2009) confirmed that consumer acceptability of cottage cheese is associated with diacetyl concentration.

Consumers who eat various foods are commonly exposed to levels of diacetyl (orthonasally and retronasally) that well exceed 0.2 ppm (Table 1).

Diacetyl is produced by some species of the lactic acid bacteria family including *Streptococcus, Leuconostoc, Lactobacillus, Pediococcus*, and *Oenococcus*. Its biological function appears to be as a substrate for regenerating NAD(P) for the microorganisms' energy needs (Bartowsky and Henschke 2004). Considerable work has been devoted to increasing the amount of diacetyl naturally occurring in some products (Baranowska 2006; Macciola and others 2008; Cruz and others 2012), and metabolic engineering strategies have been proposed to obtain strains producing larger quantities of diacetyl during fermentation (Boumerdassi and others 1997).

Diacetyl is naturally present in wine, brandy, balsamic vinegar, roasted coffee, honey, ensilage, and many other fermented foods (Grandi and others 1980; Ugliano and others 2003; Bartowsky and Henschke 2004). A number of low molecular-weight volatile compounds are generated by microorganisms from carbohydrate catabolism during fermentation of meats, including diacetyl, acetoin, butanediol, acetaldehyde, ethanol, and acetic, propionic, and butyric acids (Toldra 2004).

When present at a high concentration (exceeding 5 to 7 ppm), diacetyl is regarded by many to be undesirable in wine, whereas in the range of 1 to 4 ppm, depending on the style of wine, it is considered to contribute a desirable "buttery" or "butterscotch" character (Davis and others 1986). The sensory threshold of diacetyl in wine strongly depends upon the style and type of wine, with final diacetyl concentration lower in Chardonnay (0.2 ppm) , compared to other wines (0.9 ppm for Pinot Noir; 2.8 ppm for Cabernet Sauvignon) (Martineau and Henickkling 1995; Martineau and others 1995). The sensory perception of diacetyl in wine also depends on the presence of other compounds present in the wine (Bartowsky and others 2002a,b). Fornachon and Lloyd (1965) demonstrated that wines that had undergone malolactic fermentation contained significantly more diacetyl than wines that had not. Similarly, in beer, diacetyl gives a butterscotch, or buttery flavor to many lager beers; at a low level it can be desirable, but at high levels diacetyl is a flavor defect (O'Keefe 2004; Krogerus and others 2013).

The content of diacetyl in a product may both increase and decrease during fermentation. For instance, in the case of goat milk Jack cheese, diacetyl increased up to week 18 (because of formation of diacetyl by lactic acid bacteria) and then decreased up to week 30 (because of conversion of diacetyl into other fermentation end-products) during ripening (Attai 2009). Diacetyl content in foods is also influenced by storage. Diacetyl content decreased in soft goat milk cheese held under refrigerated and frozen storage (Park and Drake 2005). When intentionally added to lowfat ice cream, diacetyl content decreased from 1 ppm on day 1 to below solid-phase microextraction (SPME) detection limits (< 0.08 ppm) by day 14 with frozen storage (Chauhan and others 2010). Such decreases are explained by the fact that diacetyl is highly volatile, even at low temperatures. Diacetyl can also evaporate from nearly dry products. For instance, during the storage of skim milk powder, diacetyl loss closely followed the crystallization of lactose, which is influenced by relative humidity, temperature, and the presence of proteins and organic substances (Senoussi and others 1995).

Table 1–Levels of diacetyl measured in select foods.

Food	Range of diacetyl content in foods (ppm except where noted)	Reference
Butter	0.48 to 4.0	Bakirci and others (2002), Chrysan (2005)
Cottage cheese	0.02 to 4.0	Antinone and others (1994)
Cheddar cheese	0.23 to 0.76	Drake and others (2010)
Coffee	2.66 to 2.78	Daglia and others (2007)
Goat milk Jack cheese	5.97 to 13.68	Attai (2009)
Margarine	0.48 to 27.0	Rincon-Delgadillo and others (2012)
Microwave popcorn	2 to 24 ppm; 0.64 to 0.92 mg to emitted per bag	Egilman and Schilling (2012), Rosati and others (2007)
Wine	0.2 to 7.0	Davis and others (1986)
Yogurt	0.2 to 16.7	Baranowska (2006), Cheng (2010), Cruz and others (2012), Güler and others (2011)

To increase the levels of the natural buttery aroma associated with fermentation, starter distillates (SDL) are commonly used as ingredients in the formulation of many food products, including cottage cheese, margarine, salad dressings, sauces and gravies, snacks, soups, frosting mixes, vegetable oil spread, process cheese, baked goods, beverages, and sour cream (Rincon-Delgadillo and others 2012). The SDL are defined as steam distillates obtained by fermentation of a medium containing skim milk fortified with citric acid, which is fermented by specific lactic acid bacteria; SDL are GRAS (CFR 2011). Diacetyl is the primary component of SDL, while 8 other compounds are also produced (CFR 2011). SDL are available as water- or oil-based liquid or as encapsulated powder; usage in food products is limited by good manufacturing practices (Rincon-Delgadillo and others 2012). Rincon-Delgadillo and others (2012) characterized the volatile compounds of commercial SDL and quantitated levels of diacetyl and other Flavor and Extract Manufacturers Association-designated high-priority flavoring components found in 18 SDL samples and 24 selected dairy products using SPME-gas chromatography (GC)-mass spectrometry (MS). The diacetyl content ranged from 1.2 to 22000 ppm in the SDL samples and from 0.05 to 27 ppm in 22 out of 24 of the dairy products tested that listed either "natural flavor" or "natural and artificial flavor" on the labels. At least 40 other compounds were detected in the SDL and commercial food products.

Aroma Perception

When humans detect the buttery odor of diacetyl they are being exposed to and are inhaling diacetyl. Below certain levels, consumers cannot recognize (below recognition threshold) or even detect (below absolute threshold) the buttery aroma. The aroma threshold for diacetyl has been reported to range from 0.001 to 0.550 ppm, depending on the food matrix and the method of assessment, with the lowest value being reported for cheese (Smit and others 2005; Milesi and others 2010). Leksrisompong and others (2010) reported the orthonasal (sniffed) best estimate threshold of diacetyl to range from 0.005 ppm (in water and a 10% solution of fat at pH 7.0) to as high as 0.095 ppm (in soybean oil). If a food product contains diacetyl, when the food is handled, diacetyl volatiles are partially released into the environment and are inhaled, whether the familiar buttery aroma is detected or not. When chewing a product containing diacetyl, volatiles that were previously trapped within the food matrix are released into the mouth, transferred into the nasal cavity, and inhaled. The retronasal process during eating enables recognition of buttery aromatics; diacetyl molecules are concurrently inhaled and ingested.

The human nose is essentially an instrument that enables orthonasal signals to be channeled to the olfactory bulb epithelia in the brain. The buccal cavity is also involved in smelling; a passageway at the back of the throat connects to the nose, which enables "retronasal" sensations. When a human sniffs aromatic food, orthonasal data are received at the brain; when a human chews food, aromatic compounds are released, pass into the air in the mouth, and are retronasally channeled to the brain. Flavor is the combination of sensations from taste stimuli dissolved in saliva, and retronasal odor stimuli (volatile chemicals) in air delivered backwards into the nose from the mouth upon chewing and swallowing (Land 1996). Retronasal olfaction occurs during respiratory exhalation or after swallowing, and the differences in orthonasal and retronasal perception depend strongly on the physical characteristics of the aroma chemicals themselves (Diaz 2004). Whether sniffed or eaten, some vapors travel down the pharynx, trachea, and eventually into the lung bronchia. A barrier is formed between the mouth and pharynx (formed by the soft palate and the base of the tongue) to prevent aspiration of food into the airways or lungs (Hodgson and others 2003). Upon swallowing, a small volume (5 to 15 mL) of air is expelled from the nose at the time when the epiglottis momentarily closes the trachea between breaths (Land 1996). During eating, flavor molecules are not only transported through the respiratory tract, but are also absorbed and desorbed along the way (Dattratreya and others 2002).

Orthonasal sniffing can be considered more efficient than eating with respect to perception of flavor; during eating, less of the flavor that is released reaches the olfactory epithelium, compared to orthonasally (Diaz 2004). For instance, Voirol and Daget (1986) showed that a higher concentration of vanillin or citral was required by sniffing to perceive the same intensity level by sipping. The lower efficiency is because retronasal aromas are affected by salivation, surface area, enzymes, chewing, and temperature changes (Miettinen and others 2003; Diaz 2004). Individuals vary in rate of breathing, chewing, swallowing, and salivation, which affect the transport of flavors from the saliva phase to receptors (Taylor 2002). To add complexity to the issue, there is also a cognitive aspect to flavor perception (Land 1996). When panelists were instructed by Weel and others (2002) to focus only on flavor perception and to not take texture into account, significant differences in perceived flavor intensity were noted between gels of different hardness and water-holding capacity, though the levels of diacetyl or ethylbutyrate did not differ by chemical quantification.

Most food systems are complex mixtures of water, fat, carbohydrate, and protein. A variety of factors including shape, size, polarity, and affinity for water or protein influence how volatile compounds interact with the various food components. The amounts of aroma compounds delivered to the orthonasal cavity, and thus available for perception, are driven by their release from the food matrix, which is dictated by the affinity of each chemical for the matrix (van Ruth and Villeneuve 2002). The release of volatile aroma compounds from foods influences the perception

of flavor and acceptability of a food; many food components (fat, protein, water) affect release (Roberts and Acree 1996; van Ruth and Villeneuve 2002; Weel and others 2002; Odake and others 2006; Cruz and others 2012). The volatility of aroma compounds is complex; it depends on the vapor-liquid partitioning of volatile compounds, which determines the affinity of volatile molecules for the fat and aqueous phases of foods (Jo and Ahn 1999; Yackinous and Guinard 2000; van Ruth and Villeneuve 2002; Attai 2009).

Measuring Diacetyl Perception

Because of diacetyl's highly reactive volatile nature, quantitative measurement is challenging (Shibamoto 2014). Among the most common practices to measure volatile compounds in the field of food science is headspace analysis (either static or dynamic), and subsequent GC, which may be combined with other techniques such as mass spectrometry (MS) or olfactometry. In brief, volatile components are driven from the food or beverage (typically at or slightly above the body temperature of humans), "trapped" in a syringe or on a fiber, then delivered to a column, which is hooked to a detector that provides an output/measurement of the levels of the compounds of interest.

Pionnier and others (2005) tracked the kinetics of aroma compound release during model cheese consumption in order to clarify the relationships between flavor release and some oralparameters. Breathing, salivation, chewing, and swallowing were monitored while 8 human subjects consumed 5-g samples of a model cheese. The SPME-GC-MS method was useful for quantification and identification of diacetyl and several other aromatic compounds. Differences between panelists could be observed in the kinetics of aroma release, but for a given panelist, the same pattern of release was observed regardless of aroma compound studied. The linear relationships between the concentration of volatile compounds in model cheese and the respective GC peak areas were studied.

Sometimes diacetyl solutions are prepared for training human subjects in descriptive sensory analysis of dairy products; 20 to 200 ppm solutions of diacetyl are used as part of the basic Cheddar cheese flavor lexicon (Drake and others 2001; Singh and others 2003); a 100 ppm solution of diacetyl is used for cottage cheese flavor lexicon training (Drake and others 2009); and a 20 ppm solution of diacetyl is used as a reference for butter and margarine or spreads evaluation (Krause and others 2007). Gas chromatography-olfactometry (GC-O) effectively combines instrumental and human sensory analysis. This approach uses a gas chromatograph that separates the volatile components in a food. A trained panelist then sniffs the purified aroma compounds that exit from a port, and records his/her observation on a computer (Friedrich and Acree 1998). In Drake and others (2010), diacetyl concentrations were measured at levels ranging from 0.23 to 0.76 ppm in low-fat, reduced fat, and full-fat Cheddar cheeses using GC-O, coupled with GC-MS.

GC-O has also been used to study the aroma of raspberries and strawberries, where diacetyl is reported to be one of the most potent flavor compounds (Roberts and Acree 1996; Ubeda and others 2012). Roberts and Acree (1995) investigated basic aromatic release mechanisms by constructing a device called a retronasal aroma simulator (RAS) that simulates flavor release in the mouth, incorporating synthetic saliva addition, shearing at the shear rate in the mouth, air flow, and temperature regulation at 37 °C. Roberts and Acree (1996) combined RAS with GC-O; they described raspberry aroma as a combination of floral, buttery, fruity, raspberry, mushroom, maple syrup, vanilla, and green notes. Each of those descriptors was effectively related to a certain chemical compound using GC-MS, RAS, and GC-O.

Use of GC-O for diacetyl identification is not limited to the food industry. GC-O was used to identify diacetyl as the compound causing odor events at trace levels in the Llobregat River and Barcelona's treated water (Diaz and others 2004). Diacetyl was measured in the river water entering the water treatment plant in a range of 0.0009 to 0.260 ppm. Flavor profile analysis established 0.00005 ppm (0.05 ppb) as diacetyl's odor threshold concentration in water, with an odor recognition concentration as low as 0.0002 ppm (0.02 ppb).

Food structure influences the transport of volatiles into the oral and nasal cavities, while composition influences the interactions between flavor and nonflavor ingredients; thus, some have investigated flavor-matrix interactions (Land 1996; Weel and others 2002). Several methods have been developed to measure flavor concentrations in real time in the "nosespace" (orthonasal cavity) of test-persons during eating (Soeting and Heidema 1988; Lindinger and others 1998; Taylor and others 2000). To evaluate volatile flavor release in the expired air of humans during eating, several physiological and analytical constraints must be observed to obtain reliable data. Taylor and others (2000) developed an interface (MS-Nose) to sample air from the nose and ionize the volatile compounds contained therein by atmospheric pressure chemical ionization. The system allows quick and sensitive monitoring of the *in vivo* flavor release. Weel and others (2002) used MS-Nose to investigate the relation between food structure and sensory perception, using 150 ppm diacetyl in whey protein gels. The study involved both instrumental and human subjects (10 trained panelists). The time-intensity portion of the study required panelists to indicate the intensity of the diacetyl aroma at specific time points. Within a period of 90 s, including chewing and swallowing, peak nosespace diacetyl concentration was at about 25 s; human time-intensity peak detection took place between 20 and 35 s. The study demonstrated a linear relationship between flavor concentration and maximum intensity of nosespace flavor concentration and that the method used to measure nosespace concentration was sufficiently sensitive to detect differences in flavor release. While the instrumental analysis indicated no impact of gel hardness or water-holding capacity on flavor concentration, panelists perceived differences in flavor intensity. The authors concluded that the texture of gels drives perception of flavor intensity rather than the in-nose flavor concentration. This "psychophysical" aspect of sensory science suggests that the intensity of what we perceive is not a true indicator of the amount of diacetyl to which a consumer might be exposed.

Both orthonasal and retronasal contributions are involved with volatile compounds during the eating experience. Miettinen and others (2003) trained 12 panelists to evaluate diacetyl orthonasally and retronasally, and to indicate perceived diacetyl aroma in milk containing 40 ppm diacetyl and rapeseed oil (0%, 1%, 5%, 10%). In this study, panelists either sniffed samples (orthonasal) or made smooth mouth movements while the sample was in the mouth (retronasal) for 10 s prior to swallowing; actual chewing was not involved. The study demonstrated that food matrix fat content had only a minor (nonsignificant) effect on the volatility of the very polar diacetyl. Panelist scores for diacetyl intensity did not significantly differ between the 2 sensory procedures (orthonasal, retronasal) or among fat content of samples; headspace analysis of diacetyl release decreased slightly (not significantly) with increasing fat levels.

Odake and others (2006) did not use humans, but used a different model mouth system, a screw plunger, to investigate the process of retronasal olfaction or diacetyl (20 ppm) flavor release from skim and full-fat milk. With the screw plunger motion, which mimicked chewing and retronasal flavor release, diacetyl flavor release was 5 times higher than without screw plunger movement, which mimicked orthonasal flavor release. Similar to Miettinen and others (2003), the amount of the hydrophilic compound (diacetyl) released was not influenced by the fat content of the milk, with or without the plunger motion.

These studies demonstrate the importance of retronasal olfaction of diacetyl in foods where chewing is involved. When eating foods containing diacetyl, there is a significant, and potentially greater, release of diacetyl into the nosespace, regardless of whether olfactory perception of diacetyl aroma is higher than the initial olfactory perception. The act of chewing generates volatiles, which were previously trapped within the food matrix. Thus, when diacetyl is present in a food, whether consciously sniffed or simply eaten, whether a buttery aroma is perceived or not, diacetyl is partially exposed to the olfactory bulb and partially introduced to the lungs.

Diacetyl Health Effects

Occupational inhalation exposure to diacetyl

Popcorn has been a part of the American culture for centuries; the oldest ears of corn found in Mexico were about 6000 y old (Akpinar-Elci and others 2005). Although hand-popping was popular until the mid-20th century, the advent of MWs necessitated development of MW popcorn. To obtain the pleasing buttery aroma and flavor expected of "home-made" buttered popcorn, MW popcorn manufacturers have used a variety of flavorings, including acetaldehyde, acetic acid, acetoin, butyric acid, and diacetyl (FEMA 2004; NTP 2007; Ronk and others 2013).

The concern about the potential of diacetyl to cause pulmonary disease associated with MW popcorn began in May of 2000, when an unusual cluster of fixed airway obstructions was reported in workers in a MW popcorn plant in Missouri (Kreiss and others 2002; Akpinar-Elci and others 2005; Hubbs and others 2008). Eight workers who had formerly worked in the plant were reported by the Missouri Department of Health as having BO (Parmet and von Essen 2002; Kreiss and others 2002). Investigators from NIOSH (Kreiss and others 2002) 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 (2002) were measured by Kullman and others (2005). Over 100 different volatile organic compounds were isolated in the environment. Diacetyl concentration in the plant ranged from below detection limits to 98 ppm, with a mean 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) (Kullman and others 2005).

Since the first Missouri investigation, NIOSH has conducted at least 16 additional industrial hygiene and medical Health Hazard Evaluations in food production, food preparation, and flavoring manufacturing facilities related to diacetyl (CDC 2015). Investigators of an Ohio MW popcorn plant (Kanwal and Kullman 2004) measured the mean TWA (time weighted average) diacetyl air concentration in the slurry room to be 1.14 ppm. Time-weighted average exposure is calculated with the following formula (United States Department of Labor 2012):

$$\mathrm{TWA} = (C_a XT_a + C_b XT_b + \cdots C_n XT_n)/8,$$

where C is the concentration of gas; T = the time of exposure (in h) and; and 8 = hours in a workday.

The mean TWA diacetyl air concentration in the packaging area was 0.02 ppm (Kanwal and Kullman 2004). Later, Kanwal and others (2006b) 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. Investigators have concluded that corrective actions (including better ventilation, PPE, and isolation of tanks containing flavorings) reduce exposure and protect employees (Kanwal and Kullman 2004; Kanwal and others 2006b; Cummings and others 2014; Hirst and others 2014).

Collaborating investigators who conducted work on behalf of the NIOSH at MW popcorn production plants in Illinois (Sahakian and others 2003), Ohio (Kanwal and Kullman 2004), Missouri (Kanwal and others 2006b), and Montana (Kullman and Sahakian 2007) helped produce the recommendation by the ACGIH for strict limitations to occupational diacetyl exposure. A TLV-TWA of 0.01 ppm (0.04 mg/m^3) and a TLV-STEL of 0.02 ppm (0.07 mg/m^3) have been proposed by the ACGIH for occupational exposure to diacetyl (ACGIH 2012).

However, ever since the first NIOSH report, investigators have questioned whether diacetyl truly led to lung disease in MW popcorn plant employees. In a Letter to the Editor regarding Kreiss and others (2002), Taubert and others (2002) brought into question whether diacetyl alone can be singled out as a causative agent in lung ailments. Since then, numerous investigators, including those associated with the initial research, have questioned diacetyl as a single causative agent (Kreiss and others 2007; Morgan and others 2008a,b; Finley and others 2008; Galbraith and Weill 2009; Lockey and others 2009; Ronk and others 2013). Even contrasting views by single researchers have been unearthed. Lockey and others (2009) identified a NOAEL of 0.07 ppm and a LOAEL of 0.35 ppm based upon their work. Yet the same authors (Lockey and others 2009) seemed tentative in implicating diacetyl as the cause of the disease, primarily because most of the studies provide only measures of cumulative exposure, with little specific data exploring the impact of exposure duration on disease occurrence.

For comparison, Maier and others (2010) 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 (2008b).

More recently, a re-analysis was conducted on the Health Hazard Evaluation that was performed by NIOSH (Kreiss and others 2011; 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 investigators concluded that exposures to flavoring chemicals in the workplace, including diacetyl, did not produce an increased risk of abnormal spirometric findings (Ronk and others 2013).

Animal studies with diacetyl

Animal inhalation toxicity studies have also been used to investigate the hypothesis that butter flavoring vapors (BFV) cause respiratory injury when inhaled in concentrations that may occur in the workplace. Hubbs and others (2002) 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 m^3) whole-body exposure chambers. For comparison, this is similar to a 100 kg (220 lb) human being confined in a small 35 m^3 (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 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 (ACGIH 2012). 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.

In follow-up work, Hubbs and others (2008) again selected very high doses for their rat studies. A different breed (Hla(SD)CVF) of male rats were used, but they were of the same size, and methods were similar to the previous study. The low doses (75 or 99.3 ppm) were also very high: 3750 times to nearly 5000 times higher than the proposed human TLV-STEL. Concentrations of up to 365 ppm TWA were used; a single-pulse of 1800 ppm was considered a TWA of 92.9 ppm based upon a 6-h average (which calculates to breathing the incredibly high (and unrealistic) dose of 1800 ppm diacetyl for 18.6 min before being exposed to pure fresh air). The 1949 ppm treatment translates to a 6-h TWA of 92.9 ppm in the multiple high-pulse exposure. Hubbs and others (2008) based their research on what Kanwal and others (2006a) and Kreiss and others (2002) reported as peak diacetyl concentrations "in the space in a tank holding the same butter flavoring tested in the animal studies" (reported to reach 1230 ppm). Kreiss and others (2002) reported the highest TWA measured in the workplace to be 98 ppm; the tested level translates to workers inhaling vapors in the headspace above heated butter flavoring in ventilated vats (1230 ppm) for 28.8 min straight. Authors concluded that diacetyl inhalation caused epithelial necrosis at diacetyl concentrations of 224 ppm or greater. The authors (Hubbs and others 2008) concluded that the NOAEL for rats was below 100 ppm. Since levels tested in the animals are significantly higher than those typically encountered in the workplace or in the consumer environment, caution is needed in the proper interpretation of such findings.

Other animal studies similarly investigated effects on animals from exaggerated exposure levels to diacetyl. Morgan and others (2008b) studied exposure levels that began at 25 ppm (1250 times higher than the proposed human TLV-TWA) and proceeded to the highest exposure level of 1200 ppm (60000 times the proposed TLV-STEL). At these levels, many mice experienced rhinitis, became moribund, and had to be sacrificed prior to the end of the experiment. Morgan and others (2008b) evaluated the respiratory toxicity of diacetyl in male C57Bl/6 mice using "several exposure profiles relevant to workplace conditions at microwave popcorn packaging plants." Their work resulted in findings that 5-d exposures to 200 or 400 ppm diacetyl caused necrotizing rhinitis, necrotizing laryngitis and bronchitis. When exposures were reduced to 1 h/d for 4 wk at doses of 100, 200, or 400 ppm diacetyl, a reduced incidence of nasal and laryngeal toxicity was noted. Intermittent exposures to 1200 ppm diacetyl twice a day for 15 min over a 4-wk period showed similar results. Subchronic exposures to 100 ppm diacetyl 6 h/d over 12 wk caused moderate nasal injury. The use of oropharyngeal aspiration to bypass the nose caused foci of fibrohistiocytic proliferation with little or no inflammation at the junction of the terminal bronchiole and alveolar duct. It is surprising that 100 ppm, 6 h/d, for 12 wk was considered "subchronic exposure" and "clinically relevant diacetyl exposures" given the findings from workplace monitoring, which consistently indicated much lower levels of airborne diacetyl.

In a letter to the editor regarding Morgan and others (2008b), Finley and others (2008) wrote " . . . the TWA diacetyl concentrations used by Morgan and others (25 to 400 ppm) were in fact much higher than the TWA levels measured in the ABF (artificial butter flavoring) mixing rooms, where the highest airborne concentrations" were found. Finley and others (2008) also pointed out a lack of sampling duration information, which made the 100 ppm estimate neither representative nor certain that it reflects a realistic TWA value. They also doubted the ability of these animal studies to reflect typical worker exposures.

Finley and others (2008) went further to say, "it would be more accurate to state that the respiratory effects observed in Morgan and others (2008b) occurred at diacetyl concentration orders of magnitude higher than typical TWA values measured in popcorn plant mixing rooms." In other words, the animal study findings by Morgan and others (2008b) are not appropriate for formulating occupational limits and must not be transferred to the consumer setting.

Morgan and others (2008a) disagreed with Finley and others (2008) in a rebuttal letter to the editor. They defended why they selected 100 ppm diacetyl as the high concentration in this study "because in a plant where 97.9 ppm exposures were documented, about 20% of workers had airways obstruction (Kreiss and others 2002)." Morgan and others (2008a) conceded that other components of artificial butter flavoring may contribute to respiratory disease, though diacetyl has gained the most attention because it is the most prevalent. Yet relationship or association does not mean causation, and just because a compound is most prevalent does not mean that it is the cause of an ailment. Galbraith and Weill (2009), who conducted a critical review of related journal articles and Health Hazard Evaluation Reports by the NIOSH, also did not conclude that diacetyl is the causative agent of BO cases in occupational settings involving ABF, but promoted further research to establish causative agent(s).

Others have also highlighted flaws in animal studies used to drive occupational diacetyl limit recommendations. Pierce and others (2014) pointed out that the work of Lockey and others (2009) was significantly confounded by nonoccupational diacetyl exposure from cigarette smoke and hundreds of other volatile organic compounds. Lockey and others (2009) concluded that working in a popcorn production plant outside the mixing room did not appear to be associated with an increased risk of BO based upon results from FEV_1 measurements demonstrating less than the standard

20 percent FEV_1 reduction that is required for BO classification. However, work as a pre-PAPR (powdered air-purifying respirator) mixer employee, or a cumulative exposure of > 0.8 ppm-years, was associated with evidence of airways obstruction. Pierce and others (2014) reported that exposure to cigarette smoke (mainstream and second-hand) was likely to have been a significant, and unaccounted for, nonoccupational source of diacetyl exposure in all of the existing worker studies. Pierce and others (2014) concluded that the worker studies should not be used to assess the relationship between diacetyl exposure and respiratory response nor serve as a basis for proposed occupational limits. It follows, then, that these studies should not be used to decide consumer cases.

Consumer exposure to diacetyl

Diacetyl is present in a variety of foods commonly eaten by consumers and its presence in foods results from both natural occurrences as well as from the use of diacetyl as a food additive. Consumers may be exposed to diacetyl from both the oral and inhalation routes of exposure. Concentration levels of diacetyl in both food and in the air are frequently measured in terms of ppm, but such measurements are not compatible and cannot simply be combined to estimate total exposure. An exaggerated estimate of consumer dietary exposure was calculated by assuming consumers are exposed to the maximum levels shown in Table 1 for frequently-consumed foods considered to be high in diacetyl. Combining the maximum diacetyl concentrations with food consumption estimates of the various food items used by the FDA for the general U.S. population (Food and Drug Administration 2013), an average consumer of all the foods containing diacetyl at the maximum reported levels would be exposed to approximately 970 μg of diacetyl per day. Assuming a 65-kg consumer, this represents an exposure of about 15 μg/kg body weight per day.

Results of a 90-d oral toxicity study in rats indicated that daily exposures of 90 mg/kg body weight per day represented a NOAEL (Colley and others 1969). Applying a 100-fold uncertainty factor to this level to account for differences in animal to human sensitivity and intra-human sensitivity, an Acceptable Daily Intake (ADI) can be estimated to be 900 μg/kg body weight per day. As such, consumers exposed to the maximum levels of diacetyl reported on common diacetyl-containing foods, which provides an exaggeration of typical consumer exposure, are still exposed to less than 2% of the ADI levels, indicating that oral exposure to diacetyl does not pose health concerns to consumers. Such a finding is consistent with the designation by the U.S. FDA that diacetyl is GRAS, based upon the long history of its presence in food (Birkenhauer and Oliver 2003).

To estimate typical air concentrations of diacetyl to which consumers might be exposed, the Environmental Protection Agency studied emissions during the process of popping and opening bags of MW popcorn. Bags of popcorn (8 brands and 17 formulations) emitted between 0.02 an 5.8 ppb diacetyl during popping and opening; an average total of 0.779 ± 0.135 mg emitted per bag; 80% of the volatiles were emitted after the bag was opened (Rosati and others 2007). These levels are between 0.1% and 29% of the TLV-STEL value of 0.02 ppm proposed by ACGIH (2012), providing further evidence that typical consumer inhalation exposure to diacetyl from MW popcorn is not a health concern. Rosati and others (2007) reported that emissions of chemicals from a single bag of MW popcorn are very low, often within an order of magnitude of the detection of limit of GC-MS.

Nevertheless, it is possible for extreme MW popcorn consumption patterns to result in potential health concerns. A Colorado man who claimed that he developed "popcorn lung" from eating about 2 bags of MW popcorn every day for 10 y was awarded damages in 2012 (Jaslow 2012). It was further revealed that this man would frequently inhale the contents of the popcorn bags immediately after opening them (Harris 2007). Air sampling around the man's house revealed airborne levels of diacetyl allegedly consistent with levels found in MW popcorn plants (Harris 2007). Yet even the man's doctor conceded that there was no definitive causal link between the man's exposure to diacetyl and his pulmonary disease (Jaslow 2012).

Egliman and others (2011) proposed a safe level of exposure to diacetyl at or below a TWA of 1 ppb for an 8-h workday, which is well below the 0.20 ppm OEL proposed by Maier and others (2010). Later, Egilman and Schilling (2012) supported a recommended exposure limit of 5 ppb over an 8-h workday, and short-term TWA of 25 ppm over 15 min. Wallace and Veith (2011) raised stern disagreement with conclusions of Egilman and others (2011), pointing out misinterpretations of research. Wallace and Veith (2011) clarified that chemicals with similar chemical reactivity potential do not necessarily have the same toxicolocial effects. In other words, although diacetyl is highly reactive, airway site binding cannot be predicted, and short-term exposure risk may differ from long-term exposure risk. In related research, Dworak and others (2013) concluded that diacetyl is unlikely to have significant respiratory sensitization potential.Thus, it appears as though the exposure recommendations proposed by Egilman and others (2011) and by Egilman and Schilling (2012) are more restrictive than necessary.

Concluding Remarks

Numerous foods, particularly fermented foods, have been a source of consumer exposure to diacetyl for millennia. The appealing aroma characteristics of diacetyl include both orthonasal and retronasal pathways. Consumers are regularly and intermittently exposed to moderate levels of diacetyl in the diet. Dietary exposure to diacetyl under exaggerated conditions representing abnormally high levels of diacetyl in several foods still represents exposure levels far below those considered to be of toxicological significance. In addition, typical levels of airborne diacetyl resulting from MW popcorn are significantly below levels of occupational health and safety concern. The levels of diacetyl in food products are not dangerous and special warning labels are not warranted. Diacetyl, at the levels found in many foods and beverages, including MW popcorn, does not present a risk to consumers and should not be implicated as a cause of lung disease for consumers of MW popcorn or other foods.

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