

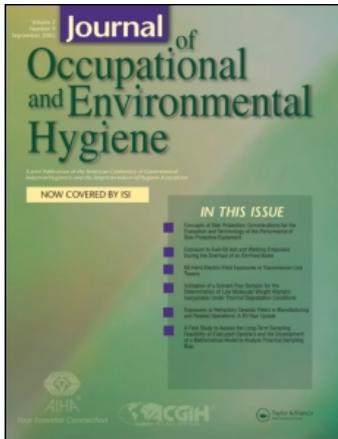
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Identification and Measurement of Diacetyl Substitutes in Dry Bakery Mix Production

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In 2008, a company using multiple buttermilk flavorings in the production of dry bakery mixes replaced one liquid flavoring containing 15–20% diacetyl with a proprietary substitute meant to lower occupational risk for diacetyl-related bronchiolitis obliterans. Subsequently, the National Institute for Occupational Safety and Health (NIOSH) evaluated buttermilk flavoring-related exposures at this company's facility, with a focus on measuring ketones by several methods. Volatile organic compounds (VOCs) were evaluated in the headspaces of six bulk flavorings samples, including the substitute buttermilk flavoring. Ketones were evaluated in workplace air via area and personal samples collected during batch preparation of the substitute buttermilk flavoring and production of a bakery mix containing the same flavoring. Air samples were evaluated using five different methods: NIOSH 2549, Modified OSHA PV2118, OSHA 1013, NIOSH Draft Procedure SMP2, and evacuated canisters. Of five buttermilk flavorings from five different flavorings manufacturers, diacetyl was present in four, including the substitute flavoring; acetoin in two; 2,3-pentanedione in four; 2,3-hexanedione in one; and 2,3-heptanedione in three. Among material safety data sheets (MSDS) for four flavorings, only one listed a hazardous ingredient, which was acetoin. The predominant flavoring ingredient identified in the headspace of the substitute flavoring was 2,3-pentanedione; all other chemicals noted above were also present. Diacetyl and 2,3-pentanedione were measured in workplace air via evacuated canisters. In one area and one personal air sample, 2,3-pentanedione was measured by OSHA Method 1013 at concentrations of 78 and 91 ppb, respectively. Without their or the employer's knowledge, workers who used buttermilk flavorings were exposed to substitute ketones from many flavorings manufacturers. Because 2,3-pentanedione, 2,3-hexanedione, and 2,3-heptanedione all share the same functional α -diketone group as diacetyl, these compounds also may share diacetyl's mechanism of toxicity. Until more is known about 2,3-pentanedione and other α -diketone compounds, they should not be assumed to be safe. Companies using artificial buttermilk flavorings should use a precautionary approach that assumes these flavorings pose a health risk and limit exposures through engineering and administrative controls and use of personal protective equipment.

Keywords 2, 3-pentanedione, alpha-diketones, bronchiolitis obliterans, exposure assessment methods

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The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

INTRODUCTION

Diacetyl, a volatile α -diketone found in butter flavoring, was first recognized as a workplace health hazard at a microwave popcorn production facility.^(1–3) Bronchiolitis obliterans, an irreversible obstructive lung disease, has since been identified throughout the microwave popcorn industry⁽⁴⁾ and in flavoring and diacetyl manufacturing workers.^(5,6) Average diacetyl exposures as low as 20 parts per billion have been measured in a microwave popcorn facility with affected workers.⁽⁴⁾ As a safe level of exposure for diacetyl is currently unknown, protecting workers from flavorings-related lung disease requires limiting exposure. Several approaches to limiting exposure have been recommended and evaluated, including engineering and administrative controls, and use of personal protective equipment (PPE); medical surveillance using spirometry is also recommended for workers exposed to diacetyl.^(7–9)

A commonly recommended engineering control for limiting exposure to a hazardous compound, and usually first on the list of hierarchical control options, is substitution. While substitution for diacetyl has been recommended as a

means of reducing health risks,⁽⁷⁾ neither the substitutes nor the health implications of substitutes have been evaluated. A commercial bakery mix production facility used multiple diacetyl-containing buttermilk flavorings in its recipes. In 2008, the facility replaced one liquid buttermilk flavoring containing 15–20% diacetyl with a proprietary substitute meant to lower the risk for diacetyl-related bronchiolitis obliterans. Subsequently, the National Institute for Occupational Safety and Health (NIOSH) received a confidential Health Hazard Evaluation request regarding the facility. Requesters' concerns included risks associated with diacetyl-containing and substitute flavorings. The goals of this study were to characterize the buttermilk flavorings used at the facility and describe exposures to flavoring ingredients using several different sampling methods during the production process.

Facility and Process Descriptions

At the time of the NIOSH evaluation, the facility produced dry bakery mixes for commercial users. The operation consisted of a "printweigh" room where dry ingredients were measured; a production room where ingredients were combined and packaged; a warehouse area where bulk materials and final products were palletized and stored; a "Quality and Regulatory Operation" (QRO) laboratory (consisting of a kitchen where bakery products were prepared and tested for quality, and an ingredients room) where colorants and liquid flavorings were measured, and offices. The printweigh room, production room, and warehouse were located in one building and the laboratory and offices in another.

In the first production step, some ingredients to be used during upcoming shifts were measured in the printweigh room. A printweigh worker measured out dry ingredients, such as sodium bicarbonate, sodium acid pyrophosphate, flavorings (including dry buttermilk flavoring reportedly containing up to 1% diacetyl), and enzymes, in approximately 32- to 100-kg batches. Some ingredients were gravity-fed, but most were transferred from storage containers using hand scoops. The printweigh room was equipped with local exhaust ventilation.

In a parallel production step, a laboratory worker measured out colorants and liquid flavorings, including buttermilk flavorings, in the ingredients room. Until mid-2008, the company used a liquid buttermilk flavoring containing 15–20% diacetyl for one product that was made every 4–6 weeks but has since substituted a reformulated flavoring for this product. Preparation of each batch of the reformulated liquid buttermilk flavoring involved transferring liquid from an approximate 13-L container via a syringe, measuring the volume of liquid in a graduated cylinder, and further transferring the measured volume from the cylinder into a 2-L container inside a ventilation hood.

Over the last decade, the company had taken increasing steps to isolate the laboratory measuring tasks. Initially, colorants and liquid buttermilk flavoring were measured in the kitchen, with dilution ventilation only, but because the colorants tended to stain surfaces throughout the kitchen, an adjacent ingredients room was established. Over time, local

exhaust ventilation was installed in the ingredients room, including a backdraft table and, most recently, an overhead capture hood. An improved respiratory protection program for laboratory workers was introduced in 2007. Half-facepiece air-purifying respirators with organic vapor cartridges were used during handling of liquid flavorings.

Production workers (mixer operators) collected batches of dry powdered flavorings from the printweigh room, transported them to the production room, and placed them into downdraft ventilated hoppers along with flour, sugar, salt, and other solid ingredients. Mixer operators poured batches of liquid flavorings into a heated shortening tank by manually opening and closing a hinged lid; this activity occurred about three times per hour over the course of an 8-hr shift. Blending of all ingredients was an automated process that took place in a closed bulk-mix delivery system; finished bake mixes were auger-fed into approximately 23-kg bags, after which the bags were heat-sealed by packer operators and sent by a conveyor belt to a palletizer. The bag filling process was equipped with local exhaust ventilation.

The facility operated 24 hr per day in three shifts. At the time of our evaluation, the work force consisted of 41 people: 27 production workers, 3 laboratory workers, 4 supervisors, 1 local manager, 1 regional manager, and 5 office workers. No obstructive spirometric abnormalities existed among 22 workers who participated in a concurrent medical survey; however, 4 (18%, a statistically significant excess in comparison to the general population) had restrictive spirometric abnormalities of unknown significance.⁽¹⁰⁾ A restrictive pattern on spirometry may indicate the presence of lung disease, such as lung scarring or fibrosis. While the significance of restrictive lung disease among flavorings-exposed workers is uncertain, there have been reports of restrictive lung disease in individuals who work with flavorings,⁽⁹⁾ suggesting that the spectrum of health effects related to flavorings may be broader than fixed obstruction.

METHODS

Industrial hygiene sampling was conducted in the fall of 2008 (initial survey) and spring of 2009 (follow-up survey) as part of a larger evaluation.⁽¹⁰⁾ Methods and results of both industrial hygiene surveys are reported here.

Key objectives of the initial sampling strategy were to collect: (1) bulk samples of flavorings, including the reformulated liquid buttermilk flavoring; (2) personal and area air samples during batch preparation of substitute liquid buttermilk flavoring; and (3) personal and area air samples during production of the bakery mix that contained the substitute liquid buttermilk flavoring

A follow-up survey was scheduled due to the presence of unanticipated substances in bulk flavorings and workplace air as determined from results of the initial survey. Key objectives of the follow-up survey were to characterize ketone exposures during (1) batch preparation of reformulated liquid buttermilk flavoring, and (2) production of the bakery mix that contained the reformulated liquid buttermilk flavoring. Multiple

TABLE I. Sampling and Analytical Methods Used during the September–October 2008 (Initial) and May 2009 (Follow-Up) Surveys

Analytes	Media/Sampler	Flow Rate (L/min)	Sample Duration (min)	Analytical Method	Objective	Survey
Volatile organic chemicals (VOCs) in bulks	Thermal desorption tube (sampled in NIOSH laboratory)	0.1	1 (liquid) 30 (powder)	Headspace analysis by gas chromatography-mass spectrometry (GC-MS)	Screening for identification	Initial only
VOCs in air	Thermal desorption tube	0.02	53 to 490	GC-MS by NIOSH 2549	Screening for identification	Initial and follow-up
Ketones in air (diacetyl, acetoin)	Sorbent tubes (silica gel 600 mg)	0.05	61 to 254	Gas chromatography-flame ionization detection (GC-FID) by Modified OSHA Method PV2118	Time-weighted average (TWA) concentrations	Initial only
Ketones in air (diacetyl, acetoin, 2,3-pentanedione, 2,3-hexanedione, 2,3-heptanedione)	Sorbent tubes (silica gel 600 mg)	0.05	53 to 264	GC-FID by OSHA Method 1013 ^A	TWA concentrations	Follow-up only
Ketones in air (diacetyl, 2,3-pentanedione, 2,3-hexanedione, 2,3-heptanedione)	Sorbent tubes (silica gel treated with o-phenylenediamine 600 mg)	0.15 0.05	53 53 to 263	Gas chromatography-nitrogen phosphorus detection by NIOSH Draft Procedure SMP2	TWA concentrations	Follow-up only
Ketones in air (diacetyl, 2,3-pentanedione)	Silonite-coated canisters (6 L)	0.08 and 0.02	51 and 410	GS-MS	TWA concentrations	Follow-up only

^AFully evaluated for diacetyl and acetoin, but not for 2,3-pentanedione, 2,3-hexanedione, or 2,3-heptanedione.

sampling and analytical methods were used to increase the likelihood of a successful outcome in quantification of select flavoring chemicals. Details on sampling methods used during the two surveys are provided in Table I.

Sampling and Analysis

Bulk Flavorings

Liquid and powdered bulk flavorings samples were collected in 50-mL polypropylene vials and shipped in refrigerated containers to the laboratory for analysis. Stainless steel thermal desorption (TD) tubes were pre-conditioned by heating at 375°C for 1–2 hr, followed by insertion into the

headspaces of the shipping vials, positioned above the bulk samples. Air was actively drawn through each TD tube at a flow rate of 0.1 L per minute for durations of 1 min (liquids) and 30 min (powders). Thermal desorption tubes contained three beds of sorbent media: Carbopack Y (90 mg), Carbopack B (115 mg), and Carboxen 1003 (150 mg). Following adsorption of VOCs, the tubes were desorbed at 300°C for 10 min using an automatic TD system interfaced directly to a gas chromatograph (GC) with a mass selective (MS) detector operating under electron ionization conditions. Such TD-GC-MS analyses produced total ion chromatograms that allowed identification and estimation of the relative abundance of VOCs in samples.

Personal and Area Air Contaminants

During the initial survey, full- and partial-shift area air samples were collected for VOC screening by NIOSH Method 2549⁽¹¹⁾ and ketones (diacetyl and acetoin) by Modified Occupational Safety and Health Administration (OSHA) Method PV2118.⁽¹²⁾ Personal samples were also collected for the ketones by Modified OSHA Method PV2118. Because sampling durations for ketones were limited to approximately 240 min, sorbent tubes for all ketone samples were collected and replaced during midshift. Sampling pumps were pre- and post-calibrated each day of sample collection. All VOC and ketone samples were stored and shipped to the laboratory in refrigerated containers either the day of or the day following sample collection and were kept out of direct sunlight. Samples were collected at different locations in the facility, including the QRO laboratory (ingredients room and kitchen), printweigh room, production room (mixing and packing areas), warehouse (palletizing area), and front offices.

Area air sampling methods for the follow-up survey included: (1) VOC screening by NIOSH Method 2549;⁽¹¹⁾ (2) ketones by OSHA Method 1013⁽¹³⁾ (diacetyl, acetoin, 2,3-pentanedione, 2,3-heptanedione, and 2,3-hexanedione); (3) ketones by NIOSH Draft Procedure SMP2 (all ketones above except acetoin); and (4) ketones using evacuated canisters (diacetyl and 2,3-pentanedione). Personal samples for ketones were collected using only OSHA Method 1013. All samples were collected at locations similar to those sampled in the initial survey and were comparably managed.

Considering recent changes in recommended air sampling methods for diacetyl,⁽¹⁴⁾ it is important to further describe the suite of methods used to sample for ketones in air during the initial and follow-up surveys. OSHA Method PV2118⁽¹²⁾ is a partially evaluated method involving the use of two sorbent tubes connected in series by flexible tubing, each containing 225 mg of silica gel. The primary modification to the method during the initial survey was to replace the tubes recommended by the method with larger tubes containing 600 mg of a specially cleaned and dried silica gel. A procedure used to clean and dry the silica gel involves placing the silica gel into deionized water, heating to boiling, and rinsing four to five times. After repeating this cleaning procedure, the silica gel is dried overnight in a 100°C oven.⁽¹⁵⁾ These modifications would, in theory, allow for increased sampling durations and improve collection efficiency. Indeed, OSHA Method 1013,⁽¹³⁾ used during the follow-up survey, specifies the use of these larger sorbent tubes containing specially cleaned and dried silica gel and is fully evaluated for diacetyl and acetoin.

The method is not fully evaluated for 2,3-pentanedione, 2,3-hexanedione, and 2,3-heptanedione; however, these additional ketones were analyzed by the laboratory from the same samples analyzed for diacetyl and acetoin. Because ketone concentrations in this workplace environment were expected to be low, sampling durations were increased from the recommended 180 min up to approximately 240 min without concern for analyte breakthrough. NIOSH Draft Procedure

SMP2, also used during the follow-up survey, involved the use of two 600-mg silica gel tubes treated with a derivatizing agent (o-phenylenediamine) and connected in series by flexible tubing. These area air samples were collected alongside samples collected using OSHA Method 1013.⁽¹³⁾ Desorption of the treated silica gel was accomplished using ethanol; analysis by gas chromatography-nitrogen-phosphorus detection allowed for quantification of diacetyl, 2,3-pentanedione, 2,3-hexanedione, and 2,3-heptanedione but not acetoin.

To help confirm results obtained by the OSHA and NIOSH methods, the evacuated canister method utilized 6-L Silonite-coated canisters (Entech Instruments, Inc., Simi Valley, Calif.) to passively collect whole air samples from specific areas within the workplace. Two canister samples were collected alongside both OSHA Method 1013⁽¹³⁾ and NIOSH Draft Procedure SMP2 samples. External flow controllers (CS1200E, Entech) regulated airflow into the canisters. Aliquots of whole-air samples were pre-concentrated (7100 Pre-concentrator, Entech) in the laboratory prior to chemical separation and analysis via GC-MS. Internal and external gas standards were used to assess the chemical concentrations of diacetyl and 2,3-pentanedione.

RESULTS

Ketones Identified in Bulk Samples

Six bulk samples were collected: the substitute liquid buttermilk flavoring, four different powdered buttermilk flavorings, and a nutmeg oil. Semiquantitative sampling data indicated the presence of ketone compounds in the headspaces of all bulk samples. Total ion chromatograms allowed identification of VOCs but did not allow quantification of masses or mass concentrations of the VOCs. Rather, the height and width of peaks appearing on total ion chromatograms allowed estimation of the relative abundance of VOCs. Thus, these bulk sampling results are referred to as "semiquantitative." Analysis of the substitute liquid buttermilk flavoring identified the following ketone compounds: diacetyl, acetoin, 2,3-pentanedione, 2,3-hexanedione, and 2,3-heptanedione (Tables II and III). The predominant ketone compound in the headspace of the substitute buttermilk flavoring sample was 2,3-pentanedione. One or more of the same compounds was identified in all four powdered buttermilk flavorings and the nutmeg oil.

Ketones Identified in Air

Air sampling was conducted over a total of 5 days. Semiquantitative sampling via NIOSH Method 2549⁽¹¹⁾ (Table IV) detected ketone compounds in daily air samples collected from several different areas of the facility, excluding office areas, the palletizing area, and the kitchen. Like the results of bulk sampling, these screening results are also semiquantitative. Total ion chromatograms produced from the analysis of TD tubes allowed identification and estimation of the relative abundance of VOCs in workplace air. Diacetyl, acetoin, 2,3-pentanedione,

TABLE II. Ketone Compounds in Bulk Headspace Samples (measured with thermal desorption tubes)

Bulk Samples	Manufacturer	Diacetyl	Acetoin	2,3-	2,3-	2,3-
				Pentanedione	Hexanedione	Heptanedione
Substitute liquid buttermilk flavoring ^A	A	X ^B	X	X	X	X
Powdered buttermilk flavoring #1	B	— ^C	—	X	—	X
Powdered buttermilk flavoring #2	C	X	—	X	—	—
Powdered buttermilk flavoring #3	D	X	X	X	—	X
Powdered buttermilk flavoring #4	E ^D	X	—	—	—	—
Nutmeg oil ^E	F ^F	—	X	X	—	—

^AOnly acetoin listed as a hazardous ingredient on MSDS.

^BKetone measured.

^CKetone not measured.

^DManufacturer unknown (no MSDS provided).

^EPredominant chemicals included furfural, terpenes, and terpene derivatives.

^FNo MSDS provided.

2,3-hexanedione, and 2,3-heptanedione were detected during batch preparation of the reformulated buttermilk flavoring in the ingredients laboratory and during production of the dry bakery mix in the production room.

The predominant ketone compound was 2,3-pentanedione in all samples analyzed by NIOSH Method 2549,⁽¹¹⁾ an observation confirmed by resampling during the follow-up survey. Identification of the presence of 2,3-pentanedione,

TABLE III. Structures and Physical Properties of Ketone Compounds in Bulk Flavorings and Workplace Air Samples

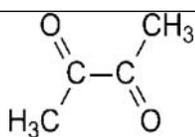
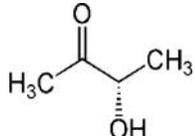
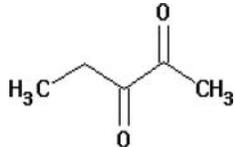
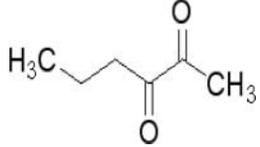
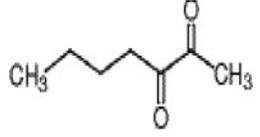
Ketone Compound	Structure	Physical Properties		
		MW (g/mol)	Density (g/cm ³)	Boiling pt. (°C)
2,3-Butanedione (Diacetyl)		86.09	0.981	88
3-Hydroxy-2-butanone (Acetoin)		88.11	1.004	148
2,3-Pentanedione (Acetyl propionyl)		100.12	0.957	108
2,3-Hexanedione (Acetyl butyryl)		114.14	0.934	128
2,3-Heptanedione (Acetyl valeryl)		128.17	0.919	144

TABLE IV. Ketone Compounds in General Area Air (measured using NIOSH Method 2549)

Activity Description	Work Area/Process Description	Date Sampled					
			Diacetyl	Acetoin	2,3-Pentanedione	2,3-Hexanedione	2,3-Heptanedione
Short-term batch preparation of the re-formulated liquid buttermilk flavoring	Ingredients lab (outside hood)	9/30/08 5/26/09	— ^A —	—	X ^B X	— —	— —
	Ingredients lab (inside hood)	9/30/08 5/26/09	X X	X —	X X	— X	X X
Production and quality testing of dry bakery mix containing the re-formulated liquid buttermilk flavoring	Packing (Line 2)	10/1/08 5/27/09	X X	— X	X X	X X	X X
	Mixing (near shortening tank)	10/1/08 5/27/09	X X	— X	X X	X X	X X
	Palletizing	10/1/08	—	—	—	—	—
	Kitchen	5/27/09	—	—	—	—	—
Short-term batch preparation of liquid nutmeg oil	Ingredients lab (outside hood)	10/2/08	—	—	—	—	—
Preparation of dry ingredients, including powdered buttermilk flavorings	Printweigh room	9/30/08 10/2/08 ^C	— —	— —	— —	— —	— —
	Packing (Line 1)	9/30/08 5/27/09	— —	— —	X X	— —	— —
Production of dry bakery mixes containing other liquid or powdered flavorings	Mixing (between dump stations)	10/2/08 5/27/09 ^D	— X	— X	X X	— X	— X
	Administrative work	Front offices	5/27/09	—	—	—	—

^AKetone not detected.

^BKetone detected.

^CSample collected, but likely contaminated prior to analysis (results not reported).

^DOn analysis, flour and possibly other dry powdered ingredients observed inside sample tube.

2,3-hexanedione, and 2,3-heptanedione during the initial survey provided necessary information for selection of ketone-specific methods to be used during the follow-up survey.

Airborne Ketone Concentrations

During the initial survey, personal air samples were collected from nine workers, one during short-term batch preparation of the substitute buttermilk flavoring and cleanup activities, and the remaining samples over an entire shift. Ten area air samples were collected, most concurrent with personal

air samples. Results for all personal and area samples (Table V) were less than the minimum detectable concentrations (MDCs) for diacetyl (47 ppb) and acetoin (93 ppb) in air. MDCs were calculated using analytical limits of detection (LODs) reported by the laboratory for diacetyl (2 µg per sample) and acetoin (4 µg per sample) divided by the volume of air sampled; sampling durations were nominally 240 min. MDCs for diacetyl and acetoin from a 74-min sample collected during batch preparation of the substitute liquid flavoring were 154 ppb and 258 ppb, respectively.

TABLE V. Air Concentrations of Ketones by Sample Type and Job or Work Area in the Initial Survey (measured using Modified OSHA Method PV2118)

Activity Description	Job or Work Area	Sample Type	Date Sampled	Diacetyl (ppb)	Acetoin (ppb)
Short-term batch preparation of the re-formulated liquid buttermilk flavoring	QRO technician	Personal	9/30/08	<MDC ^A	<MDC
Production of dry bakery mix containing the re-formulated liquid buttermilk flavoring	Ingredients lab (Inside hood)	Area	9/30/08	<MDC	<MDC
	Mixer (Line 2)	Personal	10/1/08	<MDC	<MDC
	Mixing (Near shortening tank)	Area	10/1/08	<MDC	<MDC
	Packer (Line 2)	Personal	10/1/08	<MDC	<MDC
	Packing (Line 2)	Area	10/1/08	<MDC	<MDC
	Palletizer operator	Personal	10/1/08	<MDC	<MDC
	Palletizing	Area	10/1/08	<MDC	<MDC
	Team leader	Personal	10/1/08	<MDC	<MDC
Short-term batch preparation of liquid nutmeg oil	QRO technician	Personal	10/2/08	<MDC	<MDC
Preparation of dry ingredients, including powdered buttermilk flavorings	Printweigh worker	Personal	9/30/08	<MDC	<MDC
	Printweigh room	Area	9/30/08	<MDC	<MDC
	Printweigh room	Area	10/2/08	<MDC	<MDC
Production and quality testing of dry bakery mixes containing other liquid or powdered flavorings	Packer (Line 1)	Personal	9/30/08	<MDC	<MDC
	Packing (Line 1)	Area	9/30/08	<MDC	<MDC
	Mixer (Line 1)	Personal	9/30/08	<MDC	<MDC
	Mixing (Between dump stations)	Area	10/2/08	<MDC	<MDC
	Kitchen	Area	10/2/08	<MDC	<MDC
Administrative work	Front offices	Area	10/2/08	<MDC	<MDC

^AMDC = Minimum detectable concentration in air (47 ppb for diacetyl and 93 ppb for acetoin) using nominal 240-min sampling durations.

During the follow-up survey, personal air samples were collected from 13 workers, 1 during batch preparation of the reformulated buttermilk flavoring. Area air samples were collected in seven different work areas, many concurrent with the personal samples. Production of the dry bakery mix began at approximately 10:00 a.m. (midshift) on May 27; therefore, all samples on that day were collected during the second half of the first shift (roughly 10:00 a.m. to 2:00 p.m.) and during the first half of the second shift (roughly 2:00 p.m. to 6:00 p.m.).

All personal and area sampling results analyzed by OSHA Method 1013⁽¹³⁾ were less than the MDCs for diacetyl (24 ppb), acetoin (23 ppb), 2,3-hexanedione (14 ppb), and 2,3-heptanedione (6.4 ppb). Again, MDC values were calculated using nominal sampling durations of 240 min. LODs for diacetyl, acetoin, 2,3-hexanedione, and 2,3-heptanedione were 1 μg , 1 μg , 0.8 μg , and 0.4 μg per sample, respectively. The result of one area air sample collected on May 26 at three

times the nominal flow rate (0.15 L/min) during a 53-min batch preparation of reformulated buttermilk flavoring was less than the MDCs for diacetyl (36 ppb), acetoin (35 ppb), 2,3-pentanedione (31 ppb), 2,3-hexanedione (22 ppb), and 2,3-heptanedione (10 ppb). One personal air sample collected from a first-shift packer (219 min) resulted in 91 ppb for 2,3-pentanedione; the corresponding area air sample was 78 ppb for 2,3-pentanedione (Table VI).

Results of several other samples collected in the production room (mixing and packing) were between the MDC (20 ppb) and the minimum quantifiable concentration (MQC, 69 ppb) for 2,3-pentanedione in air. The MQC for 2,3-pentanedione was calculated using the analytical limit of quantification reported by the laboratory (3.4 μg per sample) divided by the volume of air sampled.

Results of all samples analyzed using the NIOSH Draft Procedure SMP2 were less than the 240-min MDCs for

TABLE VI. Air Concentrations of 2,3-Pentanedione by Sample Type and Job or Work Area in the Follow-Up Survey (measured by OSHA Method 1013)

Activity Description	Job or Work Area	Sample Type	Date Sampled	Shift	Duration (min)	2,3- Pentane-dione (ppb)
Short-term batch preparation of the reformulated liquid buttermilk flavoring	Ingredients lab (outside hood)	Area	5/26/09	1	53	<MDC ^A
	Ingredients lab (outside hood) ^B	Area	5/26/09	1	53	<MDC
	QRO technician	Personal	5/26/09	1	58	<MDC
Production and quality testing of dry bakery mix containing the reformulated liquid buttermilk flavoring	Mixer (Line 2)	Personal	5/27/09	1	248	(53) ^C
	Mixing (near shortening tank)	Area	5/27/09	1	252	(48)
	Mixer (Line 2)	Personal	5/27/09	2	179 ^D	<MDC
	Mixing (near shortening tank)	Area	5/27/09	2	188	<MDC
	Packer (Line 2)	Personal	5/27/09	1	219	91
	Packing (Line 2)	Area	5/27/09	1	257	78
	Packer (Line 2)	Personal	5/27/09	2	206	(40)
	Packing (Line 2)	Area	5/27/09	2	192	(51)
	Palletizer operator	Personal	5/27/09	1	121 ^D	<MDC
	Palletizer operator	Personal	5/27/09	2	207	<MDC
	Team leader	Personal	5/27/09	1	254	<MDC
	Team leader	Personal	5/27/09	2	227	<MDC
Kitchen	Area	5/27/09	1,2	448	<MDC	
Production of dry bakery mixes containing other liquid or powdered flavorings	Mixer (Line 1)	Personal	5/27/09	1	128 ^D	<MDC
	Mixing (between dump stations)	Area	5/27/09	1	264	(56)
	Mixer (Line 1)	Personal	5/27/09	2	210	<MDC
	Mixing (between dump stations)	Area	5/27/09	2	192	(56)
	Packer (Line 1)	Personal	5/27/09	1	245	(42)
	Packing (Line 1)	Area	5/27/09	1	250	(59)
	Packer (Line 1)	Personal	5/27/09	2	214	<MDC
	Packing (Line 1)	Area	5/27/09	2	190	(51)
Administrative work	Front offices	Area	5/27/09	1,2	451	<MDC

^AMDC = Minimum detectable concentration in air (20 ppb using nominal 240-min sampling durations).

^BSample collected at three times the nominal flow rate.

^CValues in parentheses less than the minimum quantifiable concentration in air (69 ppb using nominal 240-min sampling durations).

^DFaulty pump.

diacetyl (2.4 ppb), 2,3-hexanedione (5.4 ppb), and 2,3-heptanedione (3.2 ppb); however, results of nearly all of the samples collected in the production room (mixing and packing) were measurable for 2,3-pentanedione, ranging from 48 to 95 ppb (Table VII). LODs for diacetyl, 2,3-pentanedione, 2,3-hexanedione, and 2,3-heptanedione reported by the laboratory were 0.1 μg , 0.1 μg , 0.3 μg , and 0.2 μg per sample, respectively. The result of the sample collected on the packing line during first shift was 95 ppb for 2,3-pentanedione (the corresponding area air sample collected using OSHA Method

1013⁽¹³⁾ resulted in 78 ppb for pentanedione). Results were less than the MDC for 2,3-pentanedione (6.1 ppb) in the ingredients room, kitchen, and front offices.

Results of the canister samples collected alongside the OSHA Method 1013⁽¹³⁾ and the NIOSH Draft Procedure SMP2 samples indicated air concentrations of 25 ppb and 113 ppb for diacetyl and 2,3-pentanedione, respectively, during batch preparation of the reformulated buttermilk flavoring (51-min sampling duration). Concentrations collected near the shortening tank during first-shift production of the dry bakery

TABLE VII. Area Air Concentrations of 2,3-Pentanedione by Work Area in the Follow-Up Survey (measured by draft NIOSH Procedure SMP2)

Activity Description	Work Area	Date Sampled	Shift	Duration (min)	2,3-Pentanedione (ppb)
Short-term batch preparation of the reformulated liquid buttermilk flavoring	Ingredients lab (outside hood)	5/26/09	1	53	<MDC ^A
Production and quality testing of dry bakery mix containing the reformulated liquid buttermilk flavoring	Mixing (near shortening tank)	5/27/09	1	253	48
	Packing (Line 2)	5/27/09	2	189	(23) ^B
		5/27/09	1	257	95
	Kitchen	5/27/09	2	193	53
Production of a dry bakery mix containing other liquid or powdered flavorings	Mixing (between dump stations)	5/27/09	1 & 2	447	<MDC
		5/27/09	1	263	56
	Packing (Line 1)	5/27/09	2	193	53
		5/27/09	1	251	62
Administrative work	Front offices	5/27/09	2	190	49
Administrative work	Front offices	5/27/09	1 & 2	451	<MDC

^AMDC = Minimum detectable concentration in air (3.2 ppb using nominal 240-min sampling durations).

^BValue in parentheses less than the minimum quantifiable concentration in air of 28 ppb based on the sampling duration.

mix were 23 ppb and 50 ppb for diacetyl and 2,3-pentanedione, respectively (250-min sampling duration). Results obtained from not only the evacuated canister method but also OSHA Method 1013⁽¹³⁾ and the NIOSH Draft Procedure SMP2 have not been fully evaluated for the analyses of all chemicals.

DISCUSSION

The risks of exposure to diacetyl have been demonstrated through workplace investigations and laboratory-based studies.^(4,6,16,17) A “safe” level of diacetyl has not been established, and even low levels of diacetyl are potentially hazardous. Substitution is often cited as the preferred approach to reducing exposures to hazardous compounds in the workplace. In efforts to reduce the risk for diacetyl-related bronchiolitis obliterans, this facility commissioned a substitute liquid buttermilk flavoring to replace one that contained 15–20% diacetyl. The MSDS for this substitute flavoring listed only acetoin as a hazardous ingredient (percentage not shown) and did not list any other chemical constituents. This investigation identified 2,3-pentanedione as the predominant ketone in the headspace of the this reformulated flavoring. The reformulated liquid buttermilk flavoring also contains diacetyl, acetoin and acetoin derivatives, 2,3-hexanedione, and 2,3-heptanedione. It is interesting to note that 2,3-pentanedione was also unexpectedly identified as a component of the nutmeg oil.

While the toxicology of diacetyl substitutes is poorly described, there is reason to be concerned that this substitute

flavoring may pose health risks similar to those of the original diacetyl-containing flavoring. All of the following butter flavoring compounds are ketones, with the common chemical characteristic among them being a carbon-oxygen double bond functional group: 2,3-butanedione (diacetyl) and acetoin are characterized by a four-carbon chain; 2,3-pentanedione (acetyl propionyl), a five-carbon chain; 2,3-hexanedione, a six-carbon chain; and 2,3-heptanedione, a seven-carbon chain (Table III).

This investigation detected each of these compounds in the air in some areas during batch preparation of the reformulated buttermilk flavoring and during production of the dry bakery mix. The most commonly detected of these ketones was 2,3-pentanedione. While in most cases the concentrations of these ketones were too low to be detected, sampling results via two different methods indicated detectable concentrations of 2,3-pentanedione for a packer and the packing area. Because 2,3-pentanedione, 2,3-hexanedione, and 2,3-heptanedione all share the same functional α -diketone group as diacetyl, these compounds likely share diacetyl’s mechanism of toxicity. Initial results from three separate research institutions describing animal studies of 2,3-pentanedione are only available in abstract form but support this assertion.^(17–19) No full-length papers are currently available. Indeed, the increasing carbon chain length would be predicted to reduce water solubility and result in deeper lung penetration and perhaps greater toxicity. Until more is known about 2,3-pentanedione and other α -diketone compounds, their use as a safe alternative to diacetyl should not be assumed.

As noted, several different sampling and analytical methods were used to identify and measure ketones in workplace air. Some methods were semiquantitative while others were quantitative. Some methods were more sensitive than others. These were important factors to consider when designing a sampling strategy and when making comparisons among the results obtained by the various methods. TD tube samples (NIOSH Method 2549)⁽¹¹⁾ were used to screen for VOCs, allowing identification and semiquantitative estimation of the relative abundances of ketones in air. Evacuated canister and sorbent tube samples (OSHA Method 1013⁽¹³⁾ and NIOSH Draft Procedure SMP2) allowed measurement of ketones in air.

Importantly, analysis of TD tube and canister samples is accomplished by direct injection into the gas chromatograph, whereas analysis of sorbent tubes utilizes solvents to desorb (dilute) ketones prior to injection. Direct injection methods are more sensitive (i.e., lower limits of detection); therefore, it was not surprising that many ketones were identified via TD tubes and that diacetyl and 2,3-pentanedione were measured via evacuated canisters. It is also important to note that NIOSH Draft Procedure SMP2 was about three times more sensitive for 2,3-pentanedione than OSHA Method 1013. Therefore, it was not surprising that a greater fraction of the side-by-side sampling results were quantifiable for 2,3-pentanedione using NIOSH Draft Procedure SMP2 compared with OSHA Method 1013 (Tables VI and VII). Results obtained for 2,3-pentanedione using three different sampling and analytical methods were comparable. Samples collected using OSHA Method 1013, NIOSH Draft Procedure SMP2, and an evacuated canister in the mixing area near the shortening tank resulted in concentrations of 48 ppb, 48 ppb, and 50 ppb, respectively. Note that the value of 48 ppb from the OSHA Method 1013⁽¹³⁾ sample was between the MDC and MQC.

CONCLUSION

Due to trade secret constraints and limitations of the OSHA Hazard Communication Standard, 29CFR1910.1200, companies that use flavorings containing diacetyl substitutes are likely unaware of the constituents and their potential toxicity. Until more is known regarding safe exposure levels for diacetyl and other chemicals in buttermilk flavorings, companies that use these flavorings should use a precautionary approach that assumes these flavorings pose a health risk and limit exposures through engineering and administrative controls and use of PPE. Process isolation, along with sufficient local exhaust and general dilution ventilation, should be implemented for any open handling of buttermilk flavorings.

In addition, company management should institute mandatory respiratory protection (as part of a respiratory protection program that complies with the OSHA Respiratory Protection Standard, 29CFR1910.134) for workers exposed to airborne flavoring chemicals or dusts. Use of full-facepiece air-purifying respirators with combination organic vapor and

particulate (P100) cartridges would be recommended. Finally, medical surveillance remains prudent for flavoring-exposed workers, despite regulatory efforts to prevent bronchiolitis obliterans by lowering diacetyl exposure.

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