Appendix A

OSHA PV2118 (Diacetyl)

Diacetyl

Related Information: Chemical Sampling - Diacetyl

Method no.:	PV2118
Control no.:	T-PV2118-01-0301-CH
Target concentration:	25 ppm (88 mg/m^3)
Procedure:	Samples are collected by drawing a known volume of air through two silica gel sampling tubes connected in series. Samples are extracted with ethyl alcohol: water (95:5) and analyzed by GC using a flame ionization detector (FID).
Recommended sampling time and sampling rate:	60 min at 0.05 L/min (3 L)
Reliable quantitation limit:	0.28 ppm (1.00 mg/m^3)
Special requirements:	Samples are collected on two silica gel tubes in series. The second tube is used as a backup for the first tube. Samples should be protected from the light after sampling.
Status of method:	Partially evaluated method. This method has been subjected to established evaluation procedures of the Method Development Team and is presented for information and trial use.
Date:	January 2003 (revised September 2006)
Chemist:	Yogi C. Shah

Chromatography Team
Industrial Hygiene Chemistry Division
OSHA Salt Lake Technical Center

1. General Discussion

1.1 Background

1.1.1 History

The purpose of this evaluation was to develop a sampling procedure for diacetyl that gave a better storage stability than did the NIOSH Method 2557, which used SKC Anasorb CMS as the sampling media[1].The NIOSH method requires that the samples be refrigerated immediately after sampling, and the analysis be performed within 7 days. A more stable sampling media was desired for OSHA samples. The following media were tested at SLTC but all gave poor storage stability: coconut shell charcoal Lot 2000, 4-*tert*-butylcatechol coated charcoal, XAD-7, and OVS-7. Silica gel tubes (150mg/75 mg) were tried next and had an average storage recovery of 94.9% for samples stored at room temperature for 14 days. A sampling train of two silica gel tubes in series was necessary because a significant amount of the diacetyl was found on the smaller, backup section of the first tube in the retention study. A second tube in series insures that all of the sample will be collected on the sampling train. The desorbing solvent of 95:5 ethyl alcohol:water with 0.25 µL/mL *p*-cymene internal standard gave an average recovery of 99.1% over the concentration range of 26.5 to 529 µg of diacetyl.

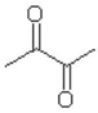
1.1.2 Toxic Effects[2] (This section is for information only and should not be taken as the basis of OSHA policy.)

In 2002, the CDC published a report in the Morbidity and Mortality Weekly Report (MMWR) on employee exposures at a microwave popcorn factory in Missouri. A group of former employees had developed fixed airways obstructive lung disease. All eight had a respiratory illness that resembled a rare lung disease called bronchiolitis obliterans. Some of the cases had such severe illness they were candidates for lung transplants. The main volatile organic chemical (VOC) found in the workplace atmospheres was diacetyl, which was used in a mixture of heated soybean oil, salt and flavorings to impart a butter flavoring to the popcorn. During NIOSH's investigation of the facility, diacetyl was chosen as a marker compound for VOC exposure. The MMWR publication reported that the geometric mean air concentration of diacetyl was 18 ppm in the room where the mixing tank was located, 1.3 ppm in the packaging area, and 0.02 in other areas of the plant. Of the eight former employees with severe respiratory illness, four were mixers and four worked in packaging. The report concluded that "workers exposed to flavorings at microwave popcorn factories are at risk for developing fixed obstructive lung disease."

1.1.3 Workplace exposure[3,4]

Diacetyl is a naturally occurring chemical in bay and other oils, beer, butter, coffee, vinegar, and other food products. It is an artificial flavoring which adds the flavor of butter, cream or creaminess, and butterscotch.

1.1.4 Physical properties and other descriptive information[3,4]

CAS number:	431-03-8	IMIS:	D740[5]
RTECS number:	EK2625000	molecular weight	86.09
melting point	-3ºC	boiling point:	88ºC
appearance:	green-yellow liquid	molecular formula:	C H O
odor:	characteristic buttery	flashpoint:	6ºC
autoignition temperature:	365ºC	density (g/mL):	0.99
solubility:	ether; alcohol; acetone. DMSO		
synonyms:	2,3 -butanedione; 2,3 -diketobutane; dimethyl diketone; dimethylglyoxal		
structure:			

This method was evaluated according to the OSHA SLTC "Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis"[6]. The Guidelines define analytical parameters, specify required laboratory tests, statistical calculations and acceptance criteria. The analyte air concentrations throughout this method are based on the recommended sampling and analytical parameters. Air concentrations listed in ppm are referenced to 25ºC and 101.3 kPa (760 mmHg).

1.2 Detection Limit of the Overall procedure (DLOP) and Reliable Quantitation Limit (RQL)

The DLOP is measured as mass per sample and expressed as equivalent air concentrations, based on the recommended sampling parameters. Ten samplers were spiked with equal descending increments of analyte, such that the highest sampler loading was 3.7 µg diacetyl. This is the amount spiked on a sampler that would produce a peak approximately 3 times the response for a sample blank. These spiked samplers were analyzed with the recommended analytical parameters, and the data obtained used to calculate the required parameters (standard error of estimate and slope) for the calculation of the DLOP. The slope was 13.89 and the SEE was 41.82. The RQL is considered the lower limit for precise quantitative measurements.

Table 1.2
Detection Limit of the Overall Procedure for Diacetyl

Mass per sample (µg)	area counts (µV-s)
0.00	293
1.3	504
1.58	631
1.85	658
2.11	676
2.38	700
2.64	734
2.90	759
3.17	788
3.43	816
3.70	838

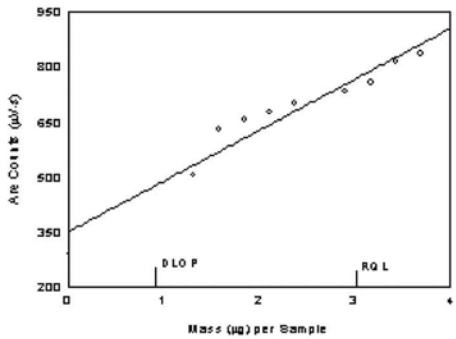


Figure 1.2.1 Plot of data to determine the DLOP/RQL for diacetyl. (Y=139X+349)

RQL is determined from the regression line parameters obtained for the calculation of the DLOP, providing 75% to 125% of the analyte is recovered. The DLOP and RQL were 0.902 µg and 3.01 µg respectively

Below is chromatogram of the RQL level.

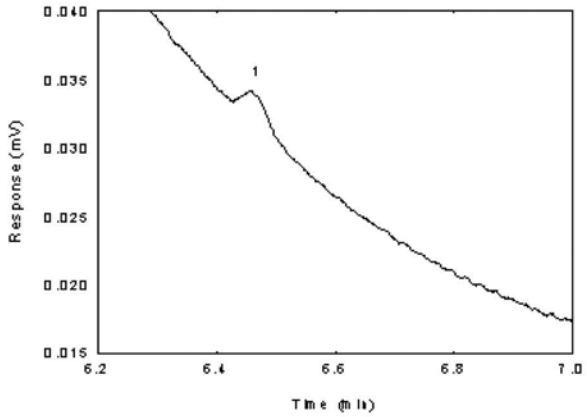


Figure 1.2.2 Chromatogram of the diacetyl standard near RQL (key: (1) diacetyl).

2. Sampling Procedure

All safety practices that apply to the work area being sampled should be followed. The sampling equipment should be attached to the worker in such a manner that it will not interfere with work performance or safety.

2.1 Apparatus

2.1.1 Samples are collected using a personal sampling pump calibrated, with the sampling device attached, to within ±5% of the recommended flow rate.

2.1.2 Silica gel tubes: glass tube with both ends flame sealed, 70 mm × 6-mm i.d. containing 2 sections of 20/40 mesh silica gel separated by a 2-mm portion of urethane foam. The adsorbing section contains 150 mg of silica gel, the backup section 75 mg. A 3-mm portion of urethane foam is placed between the outlet end of the tube and the backup section. A plug of silane-treated glass wool is placed in front of the front section(SKC No. 226-10) tubes or equivalent was used in this evaluation.

2.2 Reagents

None required.

2.3 Technique

2.3.1 Immediately before sampling, break off the ends of the flame-sealed tube to provide an opening approximately half the internal diameter of the tube. Wear eye protection when breaking ends. Use tube holders to minimize the hazard of broken glass. All tubes should be from the same lot.

2.3.2 Connect two tubes in series to the sampling pump with flexible tubing. The smaller sections of the silica gel tubes should be positioned nearer the sampling pump. The tube closer to the pump is used as a backup. A minimum amount of tubing is used to connect the two sampling tubes together. Position the sampling pump, tube holder and tubing so they do not impede work performance or safety.

2.3.3 Draw the air to be sampled directly into the inlet of the tube holder. The air being sampled is not to be passed through any hose or tubing before entering the sampling tube.

2.3.4 After sampling for the appropriate time, remove the adsorbent tube and seal it with plastic end caps. Seal each sample end-to-end with an OSHA-21 form as soon as possible.

2.3.5 Submit at least one blank sample with each set of samples. Handle the blank sample in the same manner as the other samples except draw no air through it.

2.3.6 Record sample air volumes (liters), sampling time (minutes) and sampling rate (mL/min) for each sample, along with any potential interferences on the OSHA-91A form.

2.3.7 Submit the samples to the laboratory for analysis as soon as possible after sampling. If delay is unavoidable, store the samples at refrigerator temperature. Ship any bulk samples separate from the air samples.

2.4 Extraction efficiency

The extraction efficiency was determined by liquid-spiking silica gel tubes with diacetyl at 0.1 to 2 times the target concentration. These samples were stored overnight at ambient temperature and then extracted for 30 minutes with occasional shaking and analyzed. The mean extraction efficiency over the studied range was 99.1%. The wet extraction efficiency was determined at the target concentration by liquid spiking the analyte on the front, larger, section of the first silica gel tube of the sampling train of two silica gel tubes in series, and drawing 3 L humid air (absolute humidity of 15.9 mg/L of water, about 80% relative humidity at 22.2ºC) through them. The mean recovery for the wet samples was 100.2 %

Table 2.4
Extraction Efficiency (%) of Diacetyl

level		*sample number*					
×target concn	µg per sample	1	2	3	4	5	mean
0.1	26.5	105.0	105.0	105.8	100.3	100.8	103.4
0.25	66.5	110.4	98.6	100.5	97.7	100	101.4
0.5	133	91.0	90.8	90.8	90.6	95.1	91.7
1.0	265	98.8	100.3	99.1	98.9	99.6	99.3
2.0	529	100.8	101.3	99.2	98.7	99.6	99.9
1.0 (wet)	265	104.2	101.6	99.6	93.3	102.2	100.2

2.5 Retention efficiency

Six silica gel tubes were spiked with 0.265 mg (25.ppm) of diacetyl and allowed to equilibrate for 6 h at room temperature in a drawer.. The spiked tubes were placed in series with a second unspiked silica gel tube and had 3 L humid air (absolute humidity of 15.9 mg/L of water, about 80% relative humidity at 22.2ºC) pulled through them at 0.05 L/min. The samples were extracted and analyzed. The mean retention recovery was 94.3%. There was no analyte found on the backup section of any of the tubes.

Table 2.5
Retention Efficiency (%) of Diacetyl

	sample number						
section	1	2	3	4	5	6	mean
front (a+b)	94.3	94.1	96.8	93.7	93.8	93.1	94.3
rear (a)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
rear (b)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
total	94.3	94.1	96.8	93.7	93.8	93.1	94.3

2.6 Sample storage

Nine silica gel tubes were spiked with 0.265 mg (25.ppm) of diacetyl and allowed to equilibrate for 6 h at room temperature in a drawer. The tubes were placed in series with a second unspiked silica gel tube and had 3 L humid air (absolute humidity of 15.9 mg/L of water, about 80% relative humidity at 22.2ºC) pulled through them at 0.05 L/min. Three samples were analyzed immediately, and the rest were sealed and stored at room temperature in a drawer. Three more were analyzed after 7 days of storage and the remaining three after 14 days of storage. The amounts recovered indicate good storage stability for the time period studied.

Table 2.6
Storage Test for Diacetyl (% Recovery)

	sample number			
time (days)	1	2	3	mean
0	99.4	96.9	96.2	97.5
7	94.5	97.7	95.0	95.7
14	97.2	92.8	94.8	94.9

2.7 Recommended air volume and sampling rate.

Based on the data collected in this evaluation, 3-L air samples should be collected at a sampling rate of 0.05 L/min for 60 minutes.

2.8 Interferences (sampling)

2.8.1 There are no known compounds that will severely interfere with the collection of diacetyl.

2.8.2 Suspected interferences should be reported to the laboratory with submitted samples.

3. Analytical Procedure

Adhere to the rules set down in your Chemical Hygiene Plan. Avoid skin contact and inhalation of all chemicals and review all appropriate MSDSs.

3.1 Apparatus

3.1.1 A gas chromatograph equipped with an FID. For this evaluation, an Agilent 6890 Plus gas Chromatograph equipped with a 7683 Automatic Sampler was used.

3.1.2 A GC column capable of separating diacetyl from the desorption solvent, internal standard and any potential interferences. A 60-m × 0.32-mm i.d. capillary DBWAX with a 0.5-µm df (J&W Scientific) was used in the evaluation.

3.1.3 An electronic integrator or some other suitable means of measuring peak areas. A Waters Millennium[32] Data System was used in this evaluation.

3.1.4 Amber glass vials with poly(tetrafluoroethylene)-lined caps. For this evaluation 2-mL vials were used.

3.1.5 A dispenser capable of delivering 1.0 mL of desorbing solvent to prepare standards and samples. If a dispenser is not available, a 1.0-mL volumetric pipet may be used.

3.1.7 Volumetric flasks - 10-mL and other convenient sizes for preparing standards.

3.1.8 Calibrated 10-µL syringe for preparing standards.

3.2 Reagents

3.2.1 Diacetyl, Reagent grade. Aldrich 99% (lot 09122TS BO) was used in this evaluation.

3.2.2 Ethyl alcohol, USP grade 190 proof. Aaper (lot 98G23BB) was used for this evaluation.

3.2.3 *p*-Cymene, Reagent grade. Aldrich 99% (lot 306PZ) was used in this evaluation.

3.2.4 The extraction solvent was 0.25 µL/mL *p*-cymene in ethyl alcohol:water (95:5).

3.2.5 GC grade nitrogen, air, and hydrogen.

3.3 Standard preparation

3.3.1 Prepare working analytical standards by injecting micro liter amounts of diacetyl into volumetric flasks containing the extraction solvent. An analytical standard at a concentration of 0.530 mg/mL (5.3 µL/10 mL) is equivalent to 50 ppm based on a 3-L air volume. Stock standards were stored in amber vials at refrigerated temperature for stability.

3.3.2 Bracket sample concentrations with working standard concentrations. If sample concentrations are higher than the concentration range of prepared standards, prepare and analyze additional standards, at least as high a concentration as the highest sample, to ascertain the linearity of response, or dilute the sample with extracting solvent to obtain a concentration within the existing standard range. The range of standards used in this study was from 0.00132 to 0.60 mg/mL analyze additional standards, at least as high a concentration as the highest sample, to ascertain the linearity of response, or dilute the sample with extracting solvent to obtain a concentration within the existing standard range. The range of standards used in this study was from 0.00132 to 0.60 mg/mL.

3.4 Sample preparation

3.4.1 Remove the plastic end caps from the sample tubes and carefully transfer both adsorbent sections from front tube and each section of backup tube to separate labeled 2-mL amber glass vials. Discard the glass tube and glass wool plug.

3.4.2 Add 1.0 mL of extraction solvent to each vial using the same dispenser as used for preparation of standards.

3.4.3 Immediately seal the vials with poly(tetrafluoroethylene)-lined caps.

3.4.4 Place vials on shaker and agitate for 60 minutes.

3.5 Analysis

3.5.1 Analytical conditions.

GC conditions

injector:	200°C
detector:	250°C
run time:	16 min
column gas flow:	2.5 mL/min (hydrogen)
septum purge:	1.9 mL/min (hydrogen)
injection size:	1.0 µL (10:1 split)
column:	60-m x 0.32-mm i.d. capillary DBWAX (0.5-um df)
column temperatures:	50°C for 6 min, 15°C/min to 150°C final time 3 min
retention times:	5.51 min ethyl alcohol, 6.48 min diacetyl, 12.46 min p-cymene

FID conditions

hydrogen flow:	30mL/min
air flow:	400 mL/min
makeup flow:	25 mL/min (nitrogen)

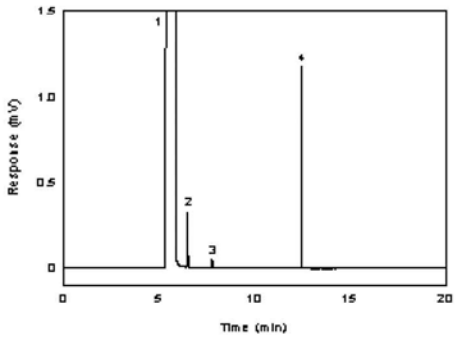


Figure 3.5.1 A chromatogram of 268 µg/ml diacetyl in 95:5 ethyl alcohol:water with 0.25 µl of p-cymene as internal standard. (Key: (1) ethyl alcohol, (2) diacetyl, (3) impurity, and (4) p-cymene).

3.5.2 Peak areas are measured by an integrator or other suitable means.

3.5.3 An internal standard (ISTD) calibration method is used. A calibration curve can be constructed by plotting ISTD-corrected response of standard injections versus micrograms of analyte per sample. Bracket the samples with freshly prepared analytical standards over a range of concentrations

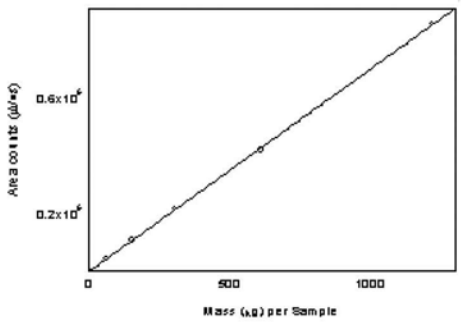


Figure 3.5.3 Calibration curve of diacetyl.
(Y = 696 x - 336)

3.6 Interferences (analytical)

3.6.1 Any compound that produces a GC response and has a similar retention time as the analyte is a potential interference. If any potential interferences were reported, they should be considered before samples are extracted. Generally, chromatographic conditions can be altered to separate an interference from the analyte.

3.6.2 When necessary, the identity or purity of an analyte peak may be confirmed by mass spectrometry or by another analytical procedure. The mass spectrum in Figure 3.6.2 was from the NIST spectral library.

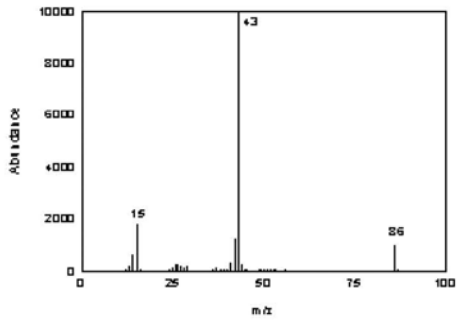


Figure 3.6.2 Mass spectrum of diacetyl.

3.6.3 Calculations

The amount of analyte per sampler is obtained from the appropriate calibration curve in terms of micrograms per sample, uncorrected for extraction efficiency. This total amount is then corrected by subtracting the total amount (if any) found on the blank. The air concentration is calculated using the following formulas.

$$C_M = \frac{M}{VE_E}$$

where C_M is concentration by weight (mg/m^3)
M is micro grams per sample
V is liters of air sampled
E_E is extraction efficiency, in decimal form

$$C_V = \frac{V_M C_M}{M_r}$$

where C_V is concentration by volume (ppm)
V_M is molar volume ate 25°C=24.46
C_M is concentration by weight
M_r is molecular weight = 86.09

4. Recommendations for Further Study

Several other tests need to be performed to make this a validated method.

1. NIOSH Method 2557.

2. Simoes E., et al. (2002) "Fixed Obstructive Lung Disease in Workers at a Microwave Popcorn Factory – Missouri" 2000-2002. MMWR 51(16):345-347.

3. O'Neil, M., *The Merck Index,* 13th ed., Merck & Co. Inc.: Whitehouse Station, NJ, 2001, p 522.

4. Lewis, R., *Sax's Dangerous Properties of Industrial Materials*, 10th ed., Vol. 2, John Wiley & Sons, New York, 2000, p 595.

5. OSHA Chemical Sampling Guide.

6. Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. C. *Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis* ; OSHA Salt Lake Technical Center, U.S. Department of Labor: Salt Lake City, UT, 1999.

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Appendix B

OSHA 1012 (Acetoin and Diacetyl)

Acetoin
Diacetyl

Method no.:	*1012*
Control no.:	*T-1012-FV-01-0811-M*
Target concentration:	*0.05 ppm (TWA) (0.18 mg/m^3) acetoin* *0.05 ppm (TWA) (0.18 mg/m^3) diacetyl*
OSHA PEL:	*none acetoin* *none diacetyl*
ACGIH TLV:	*none acetoin* *none diacetyl*
Procedure:	*Samples are collected by drawing workplace air through two tubes containing specially cleaned and dried silica gel connected in series. Samples are extracted and derivatized with a solution of 95:5 ethyl alcohol:water containing 2 mg/mL of O-(2, 3, 4, 5, 6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) and analyzed by gas chromatography using an electron capture detector (GC-ECD).*
Recommended sampling time and sampling rate:	*180 min at 0.05 L/min (9.0 L) (TWA)* *15 min at 0.2 L/min (3 L) (short term)*
Reliable quantitation limit:	*1.49 ppb (5.37 µg/m^3) acetoin* *1.30 ppb (4.57 µg/m^3) diacetyl*
Standard error of estimate at the target concentration:	*5.06% acetoin* *5.11% diacetyl*
Special requirements:	*Protect samplers from the light during and after sampling with aluminum foil or opaque tape.*
Status of method:	*Evaluated method. This method has been subjected to the established evaluation procedures of the OSHA Salt Lake Technical Center Methods Development Team.*

November 2008

Mary E. Eide

Methods Development Team
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1. *General Discussion*

For assistance with accessibility problems in using figures and illustrations presented in this method, please contact Salt Lake Technical Center (SLTC) at (801) 233-4900. This procedure was designed and tested for internal use by OSHA personnel. Mention of any company name or commercial product does not constitute endorsement by OSHA.

1.1 Background

1.1.1 History

On September 24, 2007 OSHA issued a Hazard Communication Guidance for Diacetyl and Food Flavorings Containing Diacetyl[1] in which diacetyl was identified as an indicator compound for hazardous exposures found at plants packaging microwave popcorn. This was based on Health Hazard Evaluations performed by NIOSH which found the occurrence of severe lung disease in some employees at microwave popcorn packaging plants and flavorings manufacturing facilities. In three NIOSH Health Hazard Evaluation reports, acetoin and diacetyl are listed as major constituents of butter flavoring and they were used as indicators of exposure to butter flavoring vapors.[2,3,4]

OSHA has a partially validated method for diacetyl, PV2118, which recommends the use of two standard sized silica gel tubes in series to collect diacetyl at 0.05 L/min for 1 hour.[5] There were three reasons a new method was needed: 1) the reliable quantitation limit of PV2118 is 0.28 ppm which is higher than the target concentration of 0.05 ppm for this method; 2) a new medium was needed to enable the industrial hygienist to sample for a longer sampling time and take fewer samples; and 3) to allow acetoin and diacetyl to be sampled and analyzed together. The new medium used in this method is a tube packed with specially cleaned and dried silica gel (600 mg) with a glass wool plug and a glass fiber filter in front of the dried silica gel bed (this medium is referred to as dried silica gel in this method). It was necessary to specially dry the silica gel to obtain a higher capacity because of the amount of water already present on the silica gel in the currently commercially available tubes. The dried silica gel tube can be used to sample diacetyl for up to 1.5 times longer than the currently available silica gel tube. There was not a capacity problem with acetoin. The powder and liquid formulated forms of acetoin and diacetyl may contain oily compounds and other base materials such as maltodrextin. These materials could affect the extraction of acetoin and diacetyl from the silica gel. The glass fiber filter in the tube serves only to trap these materials before they enter the silica gel bed. Retention studies using a powder containing acetoin and diacetyl showed that the acetoin and diacetyl can be stripped off the powder and collected on the silica gel, especially when sampling high humidity air. (Section 4.9)

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1 *Hazard Communication Guidance for Diacetyl and Food Flavorings Containing Diacetyl, 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dsg/guidance/diacetyl-guidance.html (accessed 3/17/2008).*

2 *HETA 2001-0474-2943 American Pop Corn Company, 2004. Centers for Disease Control and Prevention, The National Institute for Occupational Safety and Health Web site. http://www.cdc.gov/niosh/hhe/reports/pdfs/2001-0474-2943.pdf (accessed 3/15/2008).*

3 *HETA 2002-0408-2915 Agrilink Foods Popcorn Plant, 2003. Centers for Disease Control and Prevention, The National Institute for Occupational Safety and Health Web site. http://www.cdc.gov/niosh/hhe/reports/pdfs/2002-0408-2915.pdf (accessed 3/15/2008).*

4 *HETA 2003-0112-2949 ConAgra Snack Foods, 2004. Centers for Disease Control and Prevention, The National Institute for Occupational Safety and Health Web site. http://www.cdc.gov/niosh/hhe/reports/pdfs/2003-0112-2949.pdf (accessed 3/15/2008).*

5 *Shah, Y. C. OSHA Diacetyl (OSHA Method PV2118), 2003. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/partial/t-pv2118/t-pv2118.html (accessed 3/17/2008).*

To obtain adequate sensitivity for this method, it was necessary to derivatize the acetoin and diacetyl. 2,4-Dinitrophenyl hydrazine (DNPH) was the first derivatizing agent tried, but DNPH can react with both ketone and α-hydroxy ketones[6], and while it initially formed unique derivatives of acetoin and diacetyl by reacting with the first ketone group, it eventually reacted also with the alcohol group on acetoin and the second ketone group on diacetyl, forming the same derivative. In EPA Method 556.1 O-pentafluorobenzyl hydroxylamine hydrochloride (PFBHA) was used to derivatize ketone and aldehyde groups.[7] Unique derivatives of acetoin and diacetyl are formed by reacting them with PFBHA. The first ketone group on diacetyl reacts within four hours with PFBHA, but the second ketone group takes 36 hours to reach completion. Acetoin reacts within 3 hours. In this method, samples are extracted and derivatized in an extraction solution containing PFBHA. This is accomplished by first rotating the samples for 60 min and then allowing the samples to stand at room temperature for an additional 36 hours for the derivatization reaction to reach completion.

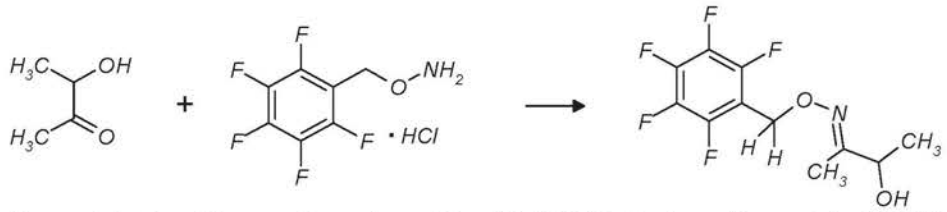

Figure 1.1.1.1. The reaction of acetoin with PFBHA to form the acetoin-PFBHA derivative.

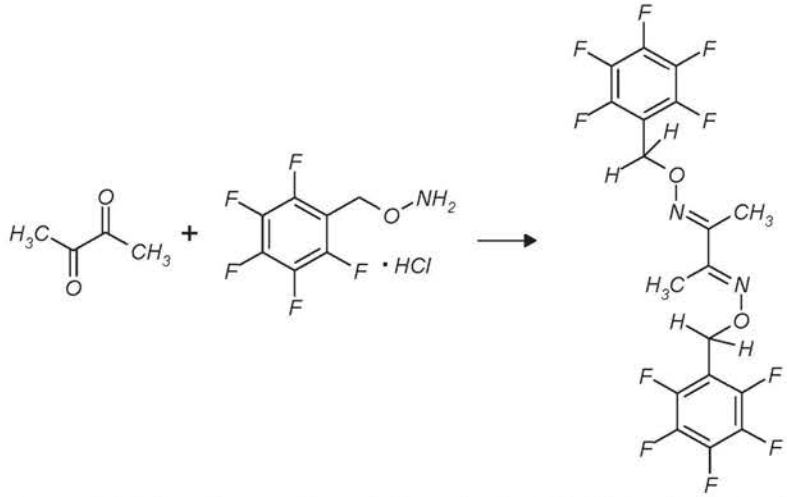

Figure 1.1.1.2. The reaction of diacetyl with PFBHA to form the diacetyl-PFBHA derivative.

This method is designed for low air concentrations of acetoin, diacetyl, and potential interferences. If high exposures are anticipated, use OSHA Method 1013[8] or increase

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[6] *Smith, M., March, J.; March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, 5th ed.; John Wiley & Sons Inc.: New York, 2001, p 1193.*

[7] *EPA Method 556.1 Determination of Carbonyl Compounds in Drinking Water by Fast Gas Chromatography, 1999. U.S. Environmental Protection Agency Web site. http://www.epa.gov/safewater/methods/pdfs/methods/met556_1.pdf (accessed 3/17/2008).*

[8] *Simmons, M., Hendricks, W. Acetoin Diacetyl (OSHA Method 1013), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1013/1013.html (accessed 11/1/2008).*

the amount of PFBHA in the extraction solution to ensure complete derivatization. Samples extracted by OSHA Method 1013 can be derivatized and analyzed by this method to detect lower concentrations.

1.1.2 Toxic effects (This section is for information only and should not be taken as the basis of OSHA policy.)

NIOSH Health Hazard Evaluations (HHE) of microwave popcorn manufacturing plants found fixed airway obstruction, in some cases, consistent with bronchiolitis obliterans in some employees.[9] Acetoin, diacetyl, acetic acid, acetaldehyde, and 2-nonanone were amongst the chemicals found by NIOSH in several popcorn manufacturing plants.[10] Diacetyl was found to be present in all workplaces where the bronchiolitis obliterans was observed, and acetoin was found in some of the workplaces. Animal toxicology studies were performed by NIOSH with diacetyl, or butter flavorings containing diacetyl. Respiratory tract damage, including necrosis of the nasal and tracheal epithelium, and death were reported in rodents exposed to diacetyl, and butter flavorings containing diacetyl, at an air concentration of approximately 200 ppm of diacetyl for 6 hours. Mice exposed to 200 and 400 ppm diacetyl via inhalation for 6 hours per day over 5 days had the following health effects: death, acute necrotizing rhinitis, and erosive or necrotizing laryngitis. Mice exposed to 200 and 400 milligrams per kilogram (mg/kg) diacetyl via oropharyngeal aspiration for 6 hours per day over 5 days had bronchiolar fibrosis and death. Rats exposed to butter flavoring vapors containing 300 ppm diacetyl for 6 hours had epithelial injury in the nasal passages and pulmonary airways.[11]

1.1.3 Workplace exposure

Workers are exposed to acetoin and diacetyl in various manufacturing processes. Acetoin and diacetyl are natural flavorings that are also synthesized for use in odor and flavor manufacturing.[12,13] Acetoin and diacetyl are found in tobacco smoke, vapors from garbage, vapors from liquid and solid animal wastes, exhaust emissions from petroleum based fuels, vapors from moldy buildings, charcoal production, vapors from latex-polyurethane backed carpet, and as chemical reagents and in chemical reactions.[14] Diacetyl is also used as an anti-microbial preservative, modifier of radiation responses for chemical and biological systems, and as a photoinitializer in polymerization of plastics.

Occupational exposure to acetoin and diacetyl in microwave popcorn manufacturing has been studied since the first reported case of severe obstructive lung disease in 2000.[15] NIOSH identified acetoin and diacetyl as useful indicator compounds that can

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[9] *Hazard Communication Guidance for Diacetyl and Food Flavorings Containing Diacetyl, 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dsg/guidance/diacetyl-guidance.html (accessed 3/17/2008).*

[10] *Flavorings-Related Lung Disease: Health Hazard Evaluations. Centers for Disease Control and Prevention, The National Institute for Occupational Safety and Health Web site. http://www.cdc.gov/niosh/topics/flavorings/hhe-eval.html (accessed 3/17/2008).*

[11] *Hazard Communication Guidance for Diacetyl and Food Flavorings Containing Diacetyl, 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dsg/guidance/diacetyl-guidance.html (accessed 3/17/2008).*

[12] *Fenarolli's Handbook of Flavor Ingredients, 5th ed.; Burdock, G.A.; CRC Press; Boca Raton, FL, 2005, p 11.*

[13] *Fenarolli's Handbook of Flavor Ingredients, 5th ed.; Burdock, G.A.; CRC Press; Boca Raton, FL, 2005, p 411.*

[14] *Chemical Information Review Document for Artificial Butter Flavoring and Constituents Diacetyl (CAS No. 431-03-8) and Acetoin (CAS No. 513-86-0), 2007. Department of Health and Human Services, National Toxicology Program Web site. http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumpdf/ Artificial_butter_flavoring.pdf (accessed 3/17/2008).*

[15] *HETA 2000-0401-2991 Gilster-Mary Lee Corporation, 2000. Centers for Disease Control and Prevention, The National Institute for Occupational Safety and Health Web site. http://www2a.cdc.gov/hhe/select.asp?PjtName=40422&bFlag=1&ID=1 (accessed 3/17/2008).*

be used to represent exposure to butter flavorings. Areas of concern were the flavor production rooms, mixing/blending rooms, packaging/production rooms, rooms where the mixing tanks were located, maintenance and cleaning operations, and quality control labs.[16]

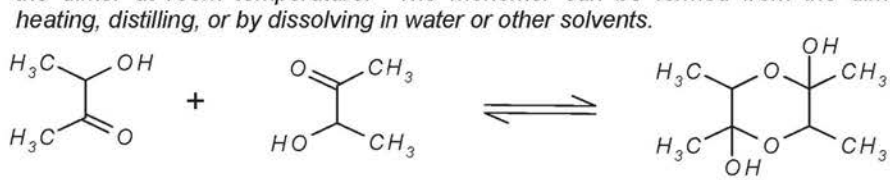
Acetoin is used as an aroma carrier and as a flavor ingredient to impart a creamy taste in fragrances and flavorings.[17] *Acetoin annual use in food and flavors manufacturing in 2004 was 34,000 pounds. Acetoin is used as a flavor ingredient for butter, milk, yogurt, and strawberry flavors. The FDA maximum allowable concentration for acetoin in beverages is 5 ppm, and in food is 50 ppm. Acetoin is naturally found in fresh apple, cooked apple, leek, cooked leek, corn, honey, cocoa, butter, roasted coffee, cheeses, yogurt, milk, wines, beer, fermented tea, scallops, crowberry, quince, and other sources. Acetoin is used in manufacturing alcoholic beverages, baked goods, breakfast cereals, cheese, chewing gum, condiments and relishes, confections and frostings, fats and oils, frozen dairy products, fruit juices, gelatins and puddings, gravies and mixes, hard candy, imitation dairy products, meat products, milk products, nonalcoholic beverages, grains, reconstituted vegetables, seasonings and flavorings, snack foods, soft candy, soups, and sweet sauce.*

Diacetyl is used as a fragrance and flavor ingredient to give products a buttery or creamy odor and flavor.[18] *Diacetyl annual use in food and flavor manufacturing in 2004 was 153,500 pounds. The FDA maximum allowable concentration for diacetyl in beverages is 5 ppm, and in food is 50 ppm. Diacetyl naturally occurs in butter, milk products, yogurt, grains, meat, wines, beer, oils of pine, oil of angelica, oils of lavender and other flowers, many flowers, raspberries, strawberries, citrus, ligonberry, guava, cabbage, peas, tomato, vinegar, cheeses, chicken, beef, mutton, pork, cognac, whiskies, tea, and coffee. Diacetyl is used in manufacturing as a flavoring in alcoholic beverages, baked goods, cheese, chewing gum, fats and oils, frozen dairy products, gelatins and puddings, gravies, hard candy, soft candy, imitation dairy, meat products, milk products, nonalcoholic beverages, and snack foods.*

1.1.4 Physical properties and other descriptive information

acetoin[19,20,21]

Acetoin is found as the liquid monomer and the solid dimer. The pure monomer forms the dimer at room temperature. The monomer can be formed from the dimer by heating, distilling, or by dissolving in water or other solvents.



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16 *HETA 2001-0474-2943 American Pop Corn Company, 2001. Centers for Disease Control and Prevention, The National Institute for Occupational Safety and Health Web site. http://www2a.cdc.gov/hhe/select.asp?PjtName=36271&bFlag=0&ID=2 (accesed 3/17/2008).*

17 *Fenarolli's Handbook of Flavor Ingredients, 5th ed.; Burdock, G.A.; CRC Press; Boca Raton, FL, 2005, p 11.*

18 *Fenarolli's Handbook of Flavor Ingredients, 5th ed.; Burdock, G.A.; CRC Press; Boca Raton, FL, 2005, p 411.*

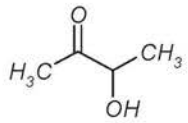
19 *Budavari, S., Ed; The Merck Index, 13th ed.; Merck & Co. Inc.: Whitehouse Station, NJ, 2001; p 68.*

20 *Material Safety Data Sheet: Acetoin, Chemwatch, Victoria, Australia (accesed 3/17/08).*

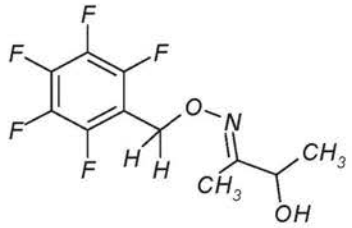
21 *Acetoin MSDS. SigmaAldrich Web site. http://www.sigmaaldrich.com/catalog/search/ProductDetail/ALDRICH/A17951 (accessed 3/17/2008).*

synonyms:	*acetyl methyl carbinol; 2,3-butanolone; 2-butanone, 3-hydoxy-; 2-butanol-3-one; dimethylketol; γ-hydroxy-β-oxobutane; 3-hydroxybutan-2-one; 3-hydroxy-2-butanone; 1-hydroxyethyl methyl ketone; methyl acetyl carbinol*
IMIS[22]:	*A624*
CAS number:	*513-86-0 (monomer); 23147-57-1 (dimer)[23]*
boiling point:	*148 °C (298 °F) (monomer)*
melting point:	*15 °C (59 °F) (monomer); 90 °C (194 °F) (dimer)*
density:	*1.005 g/mL @ 20/20 (monomer)*
molecular weight:	*88.11 (monomer)*
flash point:	*50.6 °C (123 °F) (closed cup) (monomer)*
autoignition temperature:	*370 °C (773.8 °F)*
appearance:	*clear to light yellow liquid (monomer); light cream to light yellow crystals (dimer)*
vapor density:	*>1 (air = 1)*
molecular formula:	*$C_4H_8O_2$ (monomer); $C_8H_{16}O_4$ (dimer)*
odor:	*pleasant buttery odor*
solubility:	*soluble in water; miscible with alcohol; sparingly soluble in ether and petroleum ether*
reactive hazards:	*acetoin is light sensitive [24] (Section 4.9)*

structural formula:
(monomer)



structural formula:
(acetoin-PFBHA derivative)



22 *Acetoin (OSHA Chemical Sampling Information), 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/chemicalsampling/data/CH_217010.html (accessed 3/17/2008).*

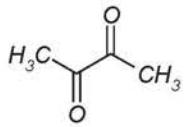
23 *CID: 90884 Acetyl Methyl Carbinol Dimer, 2008. Department of Health and Human Services, National Institutes of Health, National Center for Biotechnology Information. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=90884&loc=ec_rcs (accessed 3/17/2008).*

24 *Material Safety Data Sheet: Acetoin, 2008. The Good Scents Company Web site. http://www.thegoodscentscompany.com/msds/md102388.html (accessed 3/17/2008).*

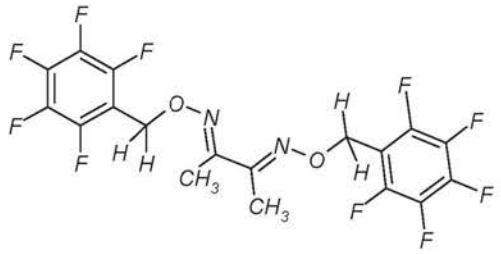
diacetyl[25,26,27,28]

synonyms:	*biacetyl; 2,3-butanedione; 2,3-butadione; 2,3-diketobutane; dimethyldiketone; dimethylglyoxal; glyoxal, dimethyl-;*
IMIS[29]*:*	*D740*
CAS number:	*431-03-8*
boiling point:	*88 °C (190 °F)*
melting point:	*3-4 °C (37.4-39.2 °F)*
density:	*0.99 g/mL @ 15/15*
molecular weight:	*86.09*
vapor pressure:	*7 kPa @ 20 °C*
flash point:	*26.7 °C (80 °F) (closed cup)*
appearance:	*yellow to yellow-green liquid*
vapor density:	*3 (air = 1)*
molecular formula:	$C_4H_6O_2$
odor:	*butter in lower concentrations, quinone odor or chlorine-like odor in higher concentrations*
solubility:	*4 parts water; miscible with alcohol, ether*
reactive hazards:	*diacetyl is light sensitive (Section 4.9); vapors may ignite when pouring or pumping due to static electricity*
autoignition temperature:	*285 °C (545 °F)*

structural formula:



structural formula:
(diacetyl-PFBHA derivative)



[25] *The Merck Index, 13th ed.; Budavari, S., Ed.; Merck & Co. Inc.: Whitehouse Station, NJ, 2001; p 522.*

[26] *Material Safety Data Sheet: Diacetyl, Chemwatch, Victoria, Australia (accessed 3/17/2008).*

[27] *Material Safety Data Sheet: 2,3-Butanedione, 2007. Fisher Scientific Web site. https://fscimage.fishersci.com/msds/03275.htm (accessed 3/17/2008).*

[28] *Material Safety Data Sheet: 2,3-Butanedione, 2007. Chem Service Inc Web site. http://www.chemservice.com/msds/msds_detail.asp?catnum=O-816 (accessed 3/17/2008).*

[29] *Diacetyl (OSHA Chemical Sampling Information), 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/chemicalsampling/data/CH_231710.html (accessed 3/17/2008).*

This method was evaluated according to the OSHA SLTC "Evaluation Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis"[30]. The Guidelines define analytical parameters, specify required laboratory tests, statistical calculations, and acceptance criteria. The analyte air concentrations throughout this method are based on the recommended sampling and analytical parameters. Air concentrations in ppm are referenced to 25 °C and 101.3 kPa (760 mmHg).

1.2 Limit defining parameters

1.2.1 Detection limit of the analytical procedure

The detection limit of the analytical procedure is 0.17 pg for acetoin and 0.11 pg for diacetyl. These are the amounts of analyte that will give a detector response that is significantly different from the response of a reagent blank. (Section 4.1)

1.2.2 Detection limit of the overall procedure

The detection limit of the overall procedure is 14.5 ng (0.447 ppb or 1.61 µg/m^3) for acetoin and 12.3 ng (0.389 ppb or 1.37 µg/m^3) for diacetyl. These are the amounts of analyte spiked on the sampler that will give detector responses that are significantly different from the responses of the respective sampler blanks. (Section 4.2)

1.2.3 Reliable quantitation limit

The reliable quantitation limit is 48.4 ng (1.49 ppb or 5.37 µg/m^3) for acetoin and 41.1 ng (1.30 ppb or 4.57 µg/m^3) for diacetyl. These are the amounts of analyte spiked on the samplers that will give detector responses that are considered the lower limits for precise quantitative measurements. (Section 4.2)

1.2.4 Instrument calibration

The standard error of estimate is 0.019 µg/sample for acetoin over the range of 0.41 to 3.28 µg/sample. The standard error of estimate is 0.052 µg/sample for diacetyl over the range of 0.40 to 3.16 µg/sample. This range corresponds to 0.25 to 2 times the TWA target concentration. (Section 4.3)

1.2.5 Precision

The precision of the overall procedure at the 95% confidence level for the ambient temperature 18-day storage test at the target concentration from dried silica gel tubes was ±9.9% for acetoin and ±10.0% for diacetyl. These each include an additional 5% for sampling pump variability. (Section 4.4)

1.2.6 Recovery

The recoveries of acetoin and diacetyl from samples used in the 18-day storage test remained above 98.4% for acetoin and 98.0% for diacetyl when the samples were stored at 23 °C. (Section 4.5)

[30] *Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M.; Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis, 1999. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/chromguide/index.html (accessed 3/15/2008).*

1.2.7 Reproducibility

Six samples were collected from a controlled test atmosphere and submitted for analysis by the OSHA Salt Lake Technical Center. The samples were analyzed according to a draft copy of this procedure after being stored at 4 °C for 20 days and at -12 °C for an additional 19 days. No individual sample result deviated from its theoretical value by more than the precision reported in Section 1.2.5. (Section 4.6)

2. Sampling Procedure

All safety practices that apply to the work area being sampled should be followed. The sampling equipment should be attached to the worker in such a manner that it will not interfere with work performance or safety.

2.1 Apparatus

Samples are collected with two tubes in series. The tubes consist of 110-cm × 7-mm o.d. glass sampling tubes packed with one section (600 mg) of specially cleaned and dried silica gel. From the front to back, the sampler consists of a silane-treated glass wool plug, glass fiber filter, 600 mg specially cleaned silica gel, and a second silane-treated glass wool plug. The silica gel should be cleaned and dried as described in Appendix A of OSHA Method 1013.[31] *The tubes used in this evaluation were labeled front and back tube. The front tube is connected to the back tube with a piece of tubing to form the sampling train. For this evaluation commercially prepared sampling tubes containing the specially dried silica gel were purchased from SKC, Inc. (Catalog no. 226-183, lot no. CPM112907-001).*

Samples are collected using a personal sampling pump calibrated, with the sampling device attached, to within ±5% of the recommended flow rate.

Use aluminum foil, opaque tape, or a tube holder, such as SKC, Inc. Cover D (catalog no. 244-29D), to protect samples from light.

2.2 Reagents

None required

2.3 Technique

Immediately before sampling, break off both ends of the flame-sealed tube to provide an opening approximately half the internal diameter of the tube. Wear eye protection when breaking the tube. Use tube holders to minimize the hazard of broken glass and to protect tubes from light exposure during sampling. All tubes should be from the same lot.

A sampling train is created by attaching two tubes in series with a small section of tubing so that the front opening of the back tube is close to the back opening of the front tube. The front of each tube contains glass wool followed by a glass fiber filter, and the back of the tube contains only the glass wool.

The back tube is used as a back-up and is positioned nearest the sampling pump. Attach the tube holder to the sampling pump so that the adsorbent tube is in an approximately vertical position with the inlet in the breathing zone. Position the sampling pump, tube holder, and tubing so they do not impede work performance or safety. Use a tube holder or wrap the tubes

[31] *Simmons, M., Hendricks, W. Acetoin Diacetyl (OSHA Method 1013), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1013/1013.html (accessed 11/1/2008).*

in aluminum foil to insure that both sampling tubes are protected from light exposure. Light will decompose the acetoin and diacetyl.

Draw the air to be sampled directly into the inlet of the tube holder. The air being sampled is not to pass through any hose or tubing before entering the sampling tube.

After sampling for the appropriate time, remove the sampling train, separate the tubes, and seal each tube with plastic end caps. Wrap each tube in aluminum foil or opaque tape, and then seal each sample end-to-end with a Form OSHA-21 seal as soon as possible.

Submit at least one blank sample with each set of samples. Handle the blank sample in the same manner as the other samples except draw no air through it.

Record sample air volumes (liters), sampling time (minutes), and sampling rate (L/min) for each sample, along with any potential interferences on the Form OSHA-91A.

Submit the samples to the laboratory for analysis as soon as possible after sampling. As a precaution, store the samples at refrigerator temperature if a delay in shipment is unavoidable. Ship any bulk samples separate from the air samples.

2.4 Sampler capacity (Section 4.7)

The sampling capacity was determined using test atmospheres containing the analytes. The concentrations of the test atmospheres were: 0.101 ppm (0.365 mg/m^3) acetoin, and 0.101 ppm (0.355 mg/m^3) diacetyl with an average relative humidity (RH) of 80% at 23 °C. The samples were collected at 0.05 L/min. The 5% breakthrough air volumes were determined to be 12.1 L for diacetyl and greater than 24 L for acetoin.

There was no acetoin or diacetyl on the back-up tube when a 15 min sample was taken at 0.2 L/min. The 5% breakthrough air volumes for a flow rate of 0.2 L/min were determined to be 11.98 L for diacetyl and greater than 13 L for acetoin.

2.5 Extraction efficiency (Section 4.8)

It is the responsibility of each analytical laboratory to determine the extraction efficiency of the analyte from the media because the adsorbent material, internal standard, reagents and laboratory techniques may be different than those listed in this evaluation and influence the results.

The mean extraction efficiencies from dry silica gel over the range of RQL to 2 times the target concentration were: 102.0% (0.022 to 3.28 µg/sample) for acetoin and 97.6% (0.01 to 3.16 µg/sample) for diacetyl. The extraction efficiency was not affected by the presence of water.

Extracted samples remain stable for at least 24 h.

2.6 Recommended sampling time and sampling rate

Sample with dried silica gel tubes for up to 180 min at 0.05 L/min (9 L) to collect TWA (long-term) samples, and for 15 min at 0.2 L/min (3 L) to collect short-term samples.

When short-term samples are collected, the air concentration equivalent to the reliable quantitation limit becomes larger. For example, the reliable quantitation limits for dried silica gel tubes for a 15 min sample taken at 0.2 L/min are 0.0044 ppm (0.016 mg/m^3) for acetoin and 0.0042 ppm (0.015 mg/m^3) for diacetyl.

2.7 Interferences, sampling (Section 4.9)

Retention efficiency

The mean retention efficiency was 96.7% for acetoin and 96.9% for diacetyl when dried silica gel tubes containing 0.819 µg of acetoin and 0.808 µg of diacetyl were allowed to sample 6.75 L of contaminant-free air having an average relative humidity of 80% at 23 °C. (Section 4.9)

Low humidity

The ability of dried silica gel tubes to collect the analytes from a relatively dry atmosphere was determined by sampling an atmosphere containing two times the target concentration and at an average relative humidity of 20% RH at 23 °C. The mean recoveries (% of theoretical) were 98.7% for acetoin and 98.5% for diacetyl. (Section 4.9)

Low concentration

The ability of dried silica gel tubes to collect the analytes at low concentrations was tested by sampling an atmosphere at 0.1 times the target concentration with at an average relative humidity of 80% RH at 23 °C. The mean recoveries (% of theoretical) were 99.0% for acetoin and 98.4% for diacetyl. (Section 4.9)

Sampling interference

The ability of dried silica gel tubes to collect the analyte when other potential interferences are present was tested under two separate series of tests. The first test was an atmosphere similar to ones found at some popcorn manufacturing plants consisting of acetoin and diacetyl at the target concentration with an interference mixture of acetaldehyde, acetic acid, and methyl ethyl ketone at an average humidity of 80% at 23 °C. All three of these interferences can react with PFBHA. The concentrations of the analytes in this test atmosphere were: 0.051 ppm (0.184 mg/m^3) acetoin and 0.051 ppm (0.180 mg/m^3) diacetyl, 1.01 ppm (1.82 mg/m^3) acetaldehyde, 1.05 ppm (2.58 mg/m^3) acetic acid, and 1.02 ppm (3.01 mg/m^3) methyl ethyl ketone. Three samplers had contaminated air drawn through them at 0.05 L/min for 180 min. All of the samples were immediately analyzed. The mean recoveries (% of theoretical) were: acetoin 97.9% and diacetyl 98.2%.

The second series of tests was with acetoin and diacetyl at the target concentration and each of the interferences listed above individually at their PEL concentration following the guidelines in SLTC "Evaluation Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis'[32]. The concentrations of these interferences are much higher than would normally be expected in a food or flavoring manufacturing workplace. The PFBHA extraction solution needed to be modified to 18 mg/mL PFBHA (72.1 µmoles/mL) to insure that there was enough PFBHA to derivatize all the analytes. These interferences and acetoin react fully within 4 hours of extraction, but the diacetyl requires 36 hours to fully react. These three test atmospheres each contained the one of the following concentrations of interference: 190 ppm (350 mg/m^3) acetaldehyde, 9.49 ppm (23.3 mg/m^3) acetic acid, or 190 ppm (560 mg/m^3) methyl ethyl ketone. These three compounds were chosen because they can collect onto the dried silica gel tubes and can react with the PFBHA. For each test, three sampling trains had contaminated air (air containing the analytes and an interference) drawn through them at 0.05 L/min for 180 min for each test. All of the samples were immediately analyzed. The average recoveries (% of theoretical) with 190 ppm acetaldehyde were 97.8% for acetoin and 95.5% for diacetyl. The average recoveries (% of theoretical) with 9.49 ppm acetic acid were 97.3% for acetoin and

[32] *Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis, 1999. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/chromguide/index.html (accessed 3/15/2008).*

98.2% for diacetyl. The average recoveries (% of theoretical) with 200 ppm methyl ethyl ketone were 98.4% for acetoin and 97.6% for diacetyl. These interferences were not a sampling interference, but under normal sample analysis, these levels of interferences would be analytical interferences. (Section 4.9)

Light

Acetoin and diacetyl are light-sensitive. The interference of light during sampling was tested using three foil-wrapped sampling trains and three uncovered sampling trains. An atmosphere containing twice the target concentration at an average relative humidity of 78% at 23°C was sampled for 180 min at 0.05 L/min, and the samples were extracted that day. The average recovery for acetoin of the foil-wrapped samplers was 98.5% and the uncovered samplers had an average recovery of 93.9%. The average recovery for diacetyl of the foil-wrapped samplers was 98.9% and the uncovered samplers had an average recovery of 94.3%. An additional three sampling trains were collected at the same time, and were protected from the light by aluminum foil. After collection, these samplers had the foil removed and were placed on the counter at ambient temperature under room light. These samples were analyzed 24 h after sampling during which they were exposed to the room light for 14 of the 24 h. The average recoveries were 81.3% for acetoin and 80.0% for diacetyl. Light is a significant interference; therefore, both tubes in the sampling train need to be covered by aluminum foil or opaque tape during and after sampling. (Section 4.9)

Powder form

The powder form of acetoin and diacetyl tested consisted of starch coated with acetoin and diacetyl. Three tests were performed on this powder. The first consisted of a sampling train of a pre-weighed PVC filter in a conical cassette in series with two dried silica gel tubes. The two dried silica gel tubes were used to collect any vapors of acetoin and diacetyl which would strip off from the powder. Known amounts of the powder were placed onto the PVC filter, and 9 L of air at an average relative humidity of 78% at 22 °C were pulled through the sampling trains at 0.05 L/min. The recovery of acetoin and diacetyl on the pre-weighed PVC filters was 0% to 1.9% for acetoin and 0% to 2.3% for diacetyl. The recovery on the dried silica gel tubes was 96.6% for acetoin and 97.8% for diacetyl. The acetoin and diacetyl recoveries were calculated from the percentages obtained from analysis of the powder and the amounts of powder weighed out. The second and third tests consisted of a sampling train of two dried silica gel tubes in series, with the powder spiked on the front glass wool of the front tube. The two tests had 9 L of air drawn through the sampling trains at 0.05 L/min, the first test used air at an average relative humidity of 20% at 22 °C, and the other test used air at an average relative humidity of 78% at 22 °C. At 20% RH most of the acetoin and diacetyl were found on the front glass wool and glass fiber filter, but at 78% RH most of the acetoin and diacetyl were found on the dried silica gel beds. These tubes can collect particulates, but cannot be used as a particulate sampler at 0.05 L/min. (Section 4.9)

3. Analytical Procedure

Adhere to the rules set down in your Chemical Hygiene Plan[33]. Avoid skin contact and inhalation of all chemicals and review all MSDSs before beginning this analytical procedure.

3.1 Apparatus

Gas chromatograph equipped with an electron capture detector. An Agilent Model 6890 GC equipped with a Chemstation, an automatic sample injector, and a μ-electron capture detector (μECD) was used in this evaluation.

[33] *Occupational Exposure to Hazardous Chemicals in Laboratories. Code of Federal Regulations, Part 1910.1450, Title 29, 2003.*

A GC column capable of separating the PFBHA derivatives of acetoin and diacetyl from the PFBHA extraction solution, potential interferences, and internal standard. A 30-m × 0.32-mm i.d. fused silica capillary column (DB-5 0.25-µm df) (Agilent Technologies, Santa Clara CA) was used in this evaluation.

An electronic integrator or other suitable means of measuring GC detector response. A Waters Empower 2 Data System was used in this evaluation.

Amber glass vials with PTFE-lined caps. Amber 2 and 4-mL vials were used in this evaluation.

A dispenser capable of delivering 2.0 mL of PFBHA extraction solution to prepare standards and samples. If a dispenser is not available, 2.0-mL volumetric pipettes can be used.

Class A volumetric flasks of appropriate sizes such as 10-mL and other convenient sizes for preparing standards.

Calibrated 10-µL syringe for preparing standards.

Micro-analytical balance capable of weighing at least 0.001 mg. An Ohaus Galaxy 160D was used in this evaluation.

Rotator. A Fisher Roto Rack was used to extract the samples.

3.2 Reagents

Acetoin, [CAS no. 513-86-0], reagent grade or better. Acetoin used in this evaluation was 99+% (lot no. 05025DH) purchased from Sigma-Aldrich (Milwaukee, WI).

Diacetyl, [CAS no. 431-03-8], reagent grade or better. Diacetyl used in this evaluation was 97% (lot no. 10815TD) purchased from Sigma-Aldrich (Milwaukee, WI).

Ethyl alcohol, [CAS no. 64-17-5], 95% v/v (190 proof) A.C.S. Spectrophotometric grade. Ethyl alcohol used in this evaluation was 95% (lot no. B0513970) purchased from Acros (Morris Plains, NJ).

O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride, [CAS no. 57981-02-9] (PFBHA), reagent grade or better. PFBHA used in this evaluation was 99+% (lot no. 1242759 54706063) purchased from Fluka, a subsidiary of Sigma-Aldrich (Milwaukee, WI).

4-Bromobenzylbromide, [CAS no. 589-15-1], reagent grade or better. 4-Bromobenzylbromide used in this evaluation was 98% (lot no. A0251708) purchased from Acros (Morris Plains, NJ).

DI water, 18 MΩ-cm. A Barnstead NanoPure Diamond system was used to purify the water for this evaluation.

The PFBHA extraction solution used for this evaluation consisted of 20 µg/mL 4-bromobenzylbromide in the 95:5 ethyl alcohol:water with 2 mg/mL PFBHA. The 4-bromobenzylbromide was added to 95:5 ethyl alcohol:water as an internal standard. Other internal standards can be used provided they are fully tested. Store this solution in a tightly sealed container in a refrigerator that does not contain solutions of aldehydes, acids, or ketones. This solution can absorb formaldehyde, other aldehydes, ketones, and acids out of the air. These compounds will react with the PFBHA, decreasing the amount available to react with acetoin or diacetyl. This solution can be stored in the refrigerator for 1 week.

3.3 Standard preparation

Prepare stock solution of acetoin and diacetyl in water. Acetoin is usually sold as the dimer, which will disassociate in water to the monomer as the solid dimer dissolves. This stock solution will remain stable for four weeks if stored in an amber bottle in the refrigerator.[34]

Freshly prepare analytical standards from the stock solutions for each analysis. These analytical standards are prepared for each of the analytes by injection of microliter amounts of a stock solution into 2-mL volumetric flasks and diluting with the PFBHA extraction solution over a concentration range of 0.02 to 6 µg/sample. For example: a target concentration standard of 1.60 µg/sample acetoin and 1.56 µg/sample diacetyl was prepared by injecting 16 µL of a stock solution containing 0.10 µg/mL acetoin and 0.10 µL/mL (0.0975 µg/mL) diacetyl in water into a 2-mL volumetric flask containing about 1.75 mL of PFBHA extraction solution and then diluting to the mark with PFBHA extraction solution (this is equivalent to 0.80 µg/mL acetoin or 0.049 ppm based on a 2-mL extraction and 9 L air volume, and 0.78 µg/mL diacetyl or 0.049 ppm based on a 2-mL extraction and 9 L air volume). Standards must be allowed to react with the PFBHA at room temperature for 36 hours.

Bracket sample concentrations with standard concentrations. If upon analysis, sample concentrations fall outside the range of prepared standards, prepare and analyze additional standards to confirm instrument response, or dilute high samples with PFBHA extraction solution and reanalyze the diluted samples.

3.4 Sample preparation

Remove the plastic end caps from the sample tube and carefully transfer the section of the adsorbent from each tube into separate 4-mL amber vials. Normally the front glass wool plug and glass fiber filter are discarded. If the industrial hygienist requests the analysis, the front glass wool plug and the glass fiber filter should be placed into a separate 4-mL amber vial. Discard the glass tubes and back glass wool plugs.

Add 2.0 mL of PFBHA extraction solution to each vial and immediately seal the vials with PTFE-lined caps.

Place the samples on a mechanical rotator and rotate at approximately 40 rpm for 60 min. Do not use a shaker to extract samples, as the recoveries will be lower.

Allow the samples to stand at room temperature for an additional 36 hours for the derivatization reaction to reach completion.

Transfer each solution from the 4-mL vial to a labeled amber 2-mL glass autosampler vial and seal with a PTFE-lined cap.

If more sensitivity is desired for samples prepared by OSHA Method 1013[35]*, they can be derivatized by the PFBHA solution and analyzed by GC-ECD. The samples in OSHA 1013 are extracted with 2 mL 95:5 ethyl alcohol:water. The samples can be derivatized by the following procedure: add 0.5-mL of sample and 0.5-mL of PFBHA extraction solution into a labeled 2-mL vial, and react for 36 hours, and then analyze by GC-ECD following the analytical conditions in this method. Standards prepared by OSHA Method 1013 are derivatized following the same procedure. The RQL will be a factor of 2 higher due to this dilution of the samples.*

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[34] *Simmons, M., Hendricks, W., Acetoin Diacetyl (OSHA Method 1013), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1013/1013.html (accessed 11/1/2008).*

[35] *Simmons, M., Hendricks, W., Acetoin Diacetyl (OSHA Method 1013), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1013/1013.html (accessed 11/1/2008).*

3.5 Analysis

3.5.1 Analytical conditions:

GC conditions:

column:	*initial 100 °C, hold 1 min, program at 5 °C/min to 200 °C, hold 0 min*
injector:	*250 °C*
detector:	*250 °C*
run time:	*20 min*
column gas flow:	*3.0 mL/min (hydrogen)*
column mode:	*constant pressure*
column pressure:	*6.8 psi*
injection size:	*1.0 µL (40:1 split)*
column:	*30-m × 0.32-mm i.d. capillary column (DB-5 df = 0.25 µm)*
retention times:	*0.85 min ethyl alcohol* *1.44 min PFBHA* *4.60 min 4-bromobenzylbromide* *5.04 min acetoin-PFBHA* *16.75 min diacetyl-PFBHA*

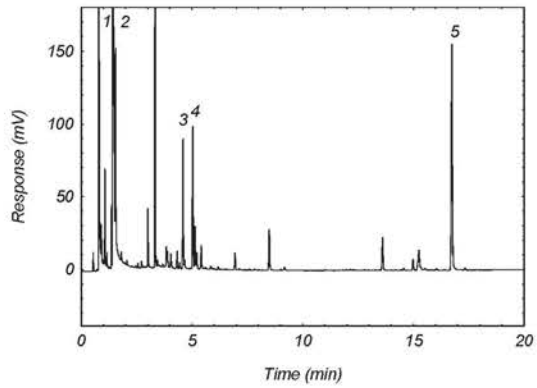

Figure 3.5.1. A chromatogram of the PFBHA derivatives of 1.60 µg/sample acetoin and 1.56 µg/sample diacetyl in the extraction solution. (Key: (1) ethyl alcohol; (2) PFBHA; (3) 4-bromobenzylbromide (ISTD); (4) acetoin-PFBHA; and (5) diacetyl-PFBHA; all other peaks are from PFBHA and its breakdown products)

ECD conditions:

makeup flow:	*40 mL/min (nitrogen)*

Peak areas are measured with an integrator or other suitable means.

3.5.2 An internal standard (ISTD) calibration method is used. A calibration curve can be constructed by plotting response of standard injections versus micrograms of analyte per sample. Bracket the samples with freshly prepared analytical standards over the range of concentrations.

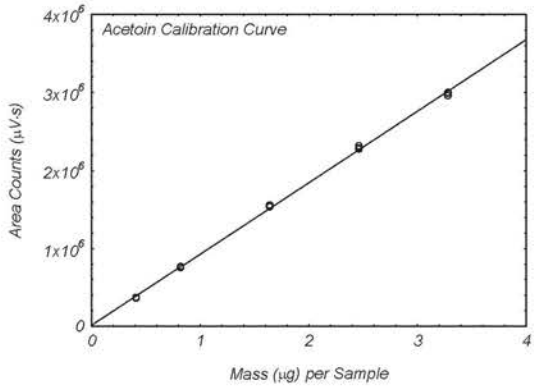

Figure 3.5.2.1. Calibration curve for acetoin. (y = 9.16E5x + 1.44E4)

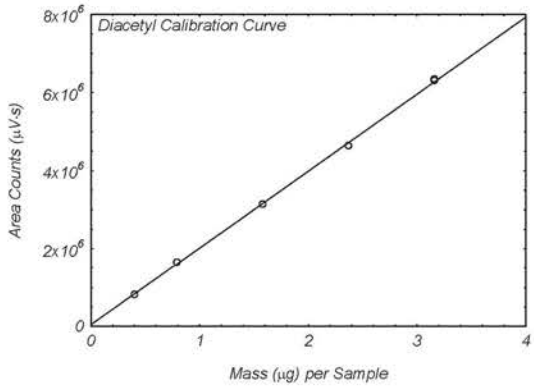

Figure 3.5.2.2. Calibration curve for diacetyl. (y = 1.97E6x + 4.59E4)

3.6 Interferences (analytical)

Any compound that produces a GC-ECD response and has a similar retention time as the analyte is a potential interference. If any potential interferences were reported, they should be considered before samples are extracted. Generally, chromatographic conditions can be altered to separate an interference from the analyte.

3.7 Calculations

The amount of analyte per sampler is obtained from the appropriate calibration curve in terms of micrograms of analyte per sample, uncorrected for extraction efficiency. The front amount found is then corrected by subtracting the total amount (if any) found on the front blank. The back amount found is then corrected by subtracting the total amount (if any) found on the back blank. The amount found on the back dried silica gel tube is added to the front tube for the total loading on each sample. The back-up tube is analyzed separately to determine the extent of analyte saturation to determine if breakthrough occurred. Even though the analytes are analyzed as the PFBHA derivatives and the calibration and results are as the amount of analyte. The air concentration is calculated using the following formulas.

$$M = [M_{front} - M_{front\ blank}] + [M_{back} - M_{back\ blank}]$$

where M is total micrograms per sample
M_{front} is micrograms found on front tube
M_{back} is micrograms found on back tube
$M_{front\ blank}$ is micrograms found on front blank tube
M_{blank} is micrograms found on back blank tube

$$C_M = \frac{M}{VE_E}$$

where C_M is concentration by weight (mg/m^3)
M is micrograms per sample
V is liters of air sampled
E_E is extraction efficiency, in decimal form

$$C_V = \frac{V_M C_M}{M_r}$$

where C_V is concentration by volume (ppm)
V_M is 24.46 (molar volume at NTP)
C_M is concentration by weight (mg/m^3)
M_r is molecular weight of analyte (acetoin = 88.11 and diacetyl = 86.09

4. Backup data

General background information about the determination of detection limits and precision of the overall procedure is found in the "Evaluation Guidelines for Air Sampling Methods Utilizing Chromatography Analysis".[36] The Guidelines define analytical parameters, specify required laboratory tests, statistical calculations, and acceptance criteria.

4.1 Detection limit of the analytical procedure (DLAP)

The DLAP is measured as the mass of analyte introduced onto the chromatographic column. Ten analytical standards were prepared with equally descending increments with the highest standard containing 97.9 ng/mL acetoin, and for diacetyl the highest standard was 95.5 ng/mL.

[36] Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis, 1999. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/chromguide/index.html (accessed 3/15/2008).

These are the concentrations that would produce peaks at least 10 times the response of a reagent blank near the elution time of the analyte. These standards, and the reagent blank were analyzed with the recommended analytical parameters (1-µL injection with a 40:1 split), and the data obtained were used to determine the required parameters (slope and standard error of estimate) for the calculation of the DLAP. For acetoin, the slope and standard error of estimate, respectively, were 3818 and 219. For diacetyl, the slope and standard error of estimate, respectively, were 9595 and 366.

Table 4.1.1
Detection Limit of the Analytical Procedure for Acetoin

concentration (ng/mL)	mass on column (pg)	area counts (µV•s)
0	0	0
9.79	0.245	863
19.6	0.490	1679
29.4	0.735	2588
39.2	0.980	3443
49.0	1.23	4167
58.7	1.47	5301
68.5	1.71	6084
78.3	1.96	7465
88.1	2.20	8098
97.9	2.45	9529

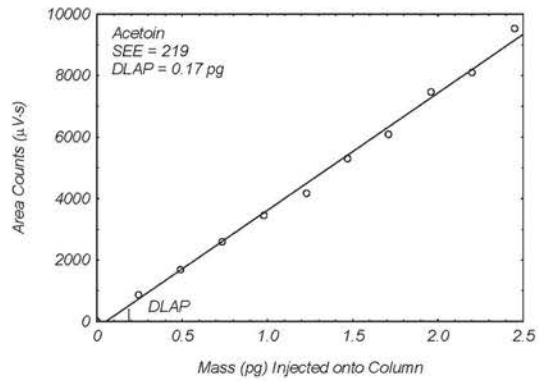

Figure 4.1.1. Plot of data to determine the DLAP for acetoin. (y = 3818x - 202)

Table 4.1.2
Detection Limit of the Analytical Procedure for Diacetyl

concentration (ng/mL)	mass on column (pg)	area counts (µV•s)
0	0	0
9.55	0.238	2824
19.1	0.478	5099
28.7	0.718	7020
38.2	0.955	9587
47.8	1.20	11701
57.3	1.43	13790
66.9	1.67	15745
76.4	1.91	18523
86.0	2.15	20511
95.5	2.39	23882

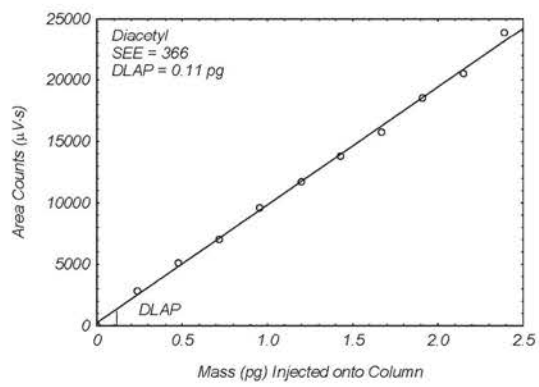

Figure 4.1.2. Plot of data to determine the DLAP for diacetyl. (y = 9595x + 238)

4.2 Detection limit of the overall procedure (DLOP) and reliable quantitation limit (RQL)

DLOP is measured as mass per sample and expressed as equivalent air concentrations, based on the recommended sampling parameters. Ten samplers were spiked with equally descending increments of analyte. The highest amount is the amount spiked on the sampler that would produce a peak approximately 10 times the response of a sample blank. These spiked samplers and the sample blank were analyzed with the recommended analytical parameters, and the data obtained used to calculate the required parameters (slope and standard error of estimate) for the calculation of the DLOP. For acetoin, the slope and standard error of estimate, respectively, were 46.9 and 227. For diacetyl, the slope and

standard error of estimate, respectively, were 121 and 497. For acetoin, the DLOP was 14.5 ng and the RQL was 48.4 ng. For diacetyl, the DLOP was 12.3 ng and the RQL was 41.1 ng.

Table 4.2.1
Detection Limit of the Overall Procedure for Acetoin

mass per sample (ng)	area counts (µV•s)
0	0
19.6	866
39.2	1901
58.7	2927
78.3	3421
97.9	4158
117	5543
137	6002
157	7399
176	8221
196	9373

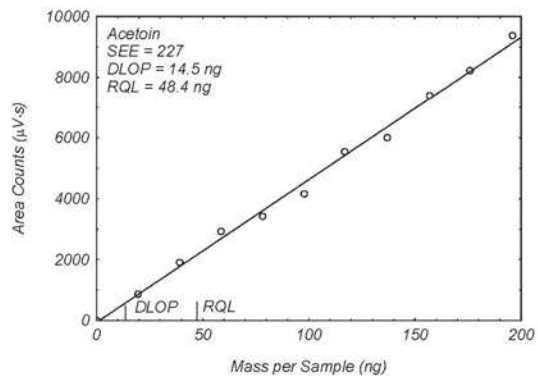

Figure 4.2.1. Plot of data to determine the DLOP/RQL for acetoin. (y = 46.9x - 63.1)

Table 4.2.2
Detection Limit of the Overall Procedure for Diacetyl

mass per sample (ng)	area counts (µV•s)
0.0	0
19.1	2758
38.2	5554
57.4	7690
76.4	10101
95.5	11743
115	13988
134	15701
153	18651
172	21621
191	23995

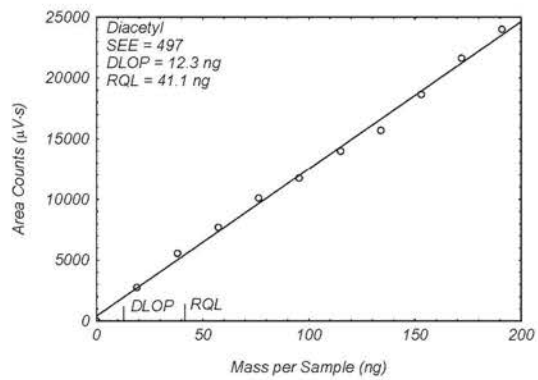

Figure 4.2.2. Plot of data to determine the DLOP/RQL for diacetyl. (y = 121x + 407)

The RQL is considered the lower limit for precise quantitative measurements. It is determined from the regression line parameters obtained for the calculation of the DLOP, providing 75% to 125% of the analyte is recovered. The RQLs are listed in Table 4.2.3.

Table 4.2.3
Reliable Quantitation Limits

analyte	ng	ppb	µg/m^3	E_E
acetoin	48.4	1.49	5.37	102.3
diacetyl	41.1	1.30	4.57	97.3

E_E = extraction efficiency

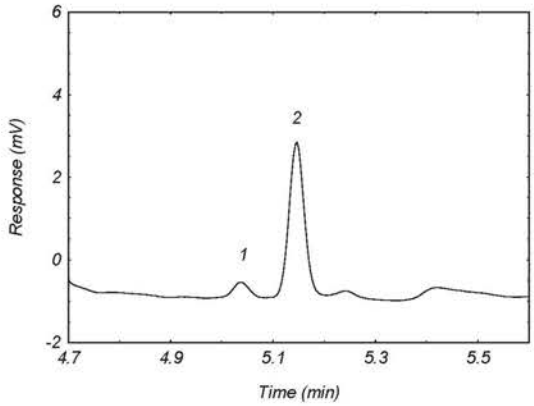

Figure 4.2.3. A chromatogram of the RQL of acetoin. (Key: (1) acetoin-PFBHA, (2) interference)

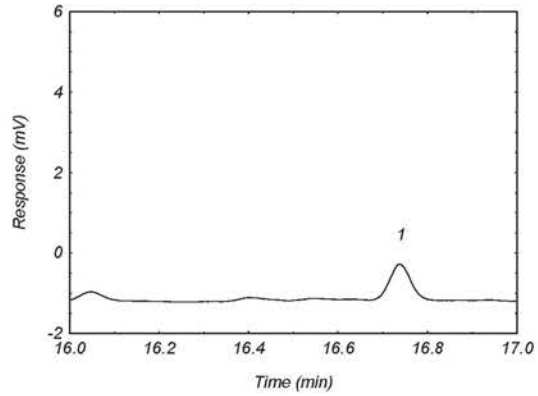

Figure 4.2.4. A chromatogram of the RQL of diacetyl. (Key: (1) diacetyl-PFHBA)

4.3 Instrument calibration

The standard error of estimate was determined from the linear regression of data points from standards over a range that covers 0.25 to 2 times the TWA target concentration. Calibration curves were constructed and shown in Section 3.5.2 from the three injections each of five standards. The standard errors of estimates were 0.019 µg for acetoin and 0.052 µg for diacetyl.

Table 4.3.1
Instrument Calibration for Acetoin

standard concn (µg/sample)	area counts (µV·s)		
0.41	367186	360667	370276
0.82	759141	752935	771533
1.64	1550965	1559979	1538639
2.46	2318162	2277568	2290341
3.28	2993893	2999180	2959244

Table 4.3.2
Instrument Calibration for Diacetyl

standard concn (µg/sample)	area counts (µV·s)		
0.40	818644	817236	817895
0.79	1658619	1654024	1658622
1.58	3140780	3142807	3140857
2.37	4604360	4645231	4644018
3.16	6349382	6315236	6309791

4.4 Precision (overall procedure)

The precision at the 95% confidence level is obtained by multiplying the standard error of estimate by 1.96 (the z-statistic from the standard normal distribution at the 95% confidence level). In Section 4.5, 95% confidence intervals are drawn about their respective regression lines in the storage graph figures. The precisions of the overall procedure were obtained from the ambient temperature 18 day storage tests were ±9.9% for acetoin and ±10.0% for diacetyl.

4.5 Storage test

Storage samples for acetoin and diacetyl were prepared using dried silica gel tubes from controlled test atmospheres using the recommended sampling conditions. The concentrations were 0.051 ppm (0.184 mg/m^3) acetoin and 0.050 ppm (0.180 mg/m^3) diacetyl at an average relative humidity of 80% at 23 °C. Thirty-three storage samples were prepared. Three samples were analyzed on the day of generation. Fifteen of the tubes were stored at reduced temperature (4 °C) and the other fifteen were stored in a closed drawer at ambient temperature (about 23 °C). At 3 to 4-day intervals, three samples were selected from each of the two storage sets and analyzed. Recoveries are not corrected for extraction efficiency.

Table 4.5.1
Storage Test for Acetoin at 80% RH

time (days)	ambient storage recovery (%)			refrigerated storage recovery (%)		
0	100.4	98.5	101.1			
4	99.1	100.3	98.9	100.1	100.4	98.6
7	99.5	99.1	98.6	98.9	99.7	100.8
10	100.5	98.8	99.4	98.5	100.1	99.9
14	97.9	99.3	98.3	99.9	99.3	98.6
18	98.5	99.3	97.6	99.8	98.3	99.1

Table 4.5.2
Storage Test for Diacetyl at 80% RH

time (days)	ambient storage recovery (%)			refrigerated storage recovery (%)		
0	100.2	100.4	98.2			
4	99.3	100.1	98.1	99.4	100.1	97.3
7	99.8	98.7	97.2	100.3	99.3	97.1
10	97.3	99.8	98.9	97.5	100.0	99.8
14	99.7	99.1	97.6	99.7	98.9	96.6
18	98.7	97.7	96.8	98.6	97.7	96.5

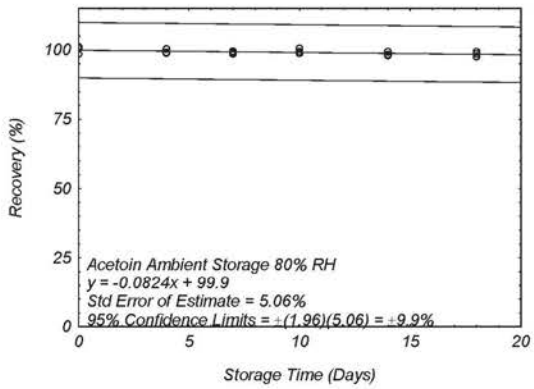

Figure 4.5.1. Ambient storage test for acetoin at 80% RH.

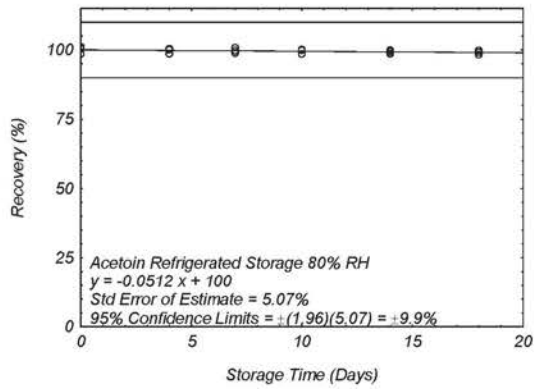

Figure 4.5.2. Refrigerated storage test for acetoin at 80% RH.

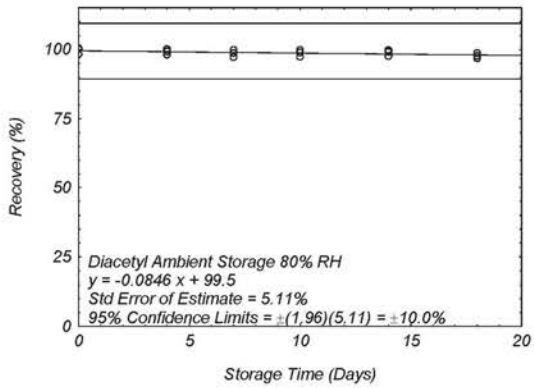

Figure 4.5.3. Ambient storage test for diacetyl at 80% RH.

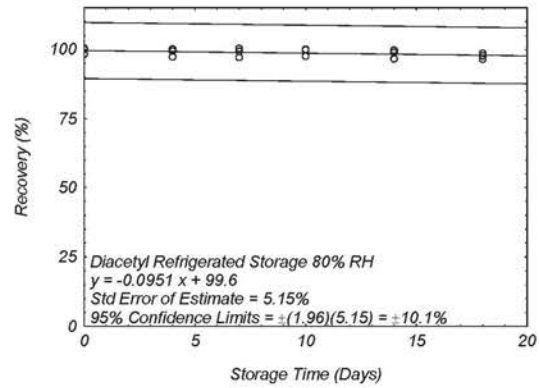

Figure 4.5.4. Refrigerated storage test for diacetyl at 80% RH.

Storage studies were also performed using tubes packed with 400/200 mg sections of dried silica gel, at an average relative humidity of 22% RH at 23 °C to determine the effects of low humidity on storage and on migration. The concentrations were 0.051 ppm (0.184 mg/m^3) acetoin and 0.050 ppm (0.180 mg/m^3) diacetyl. Thirty-three storage samples were prepared. Three samples were analyzed on the day of generation. At 3 to 4-day intervals, three samples were selected from each of the two storage sets and analyzed. Fifteen of the tubes were stored at reduced temperature (4 °C) and the other fifteen were stored in a closed drawer at ambient temperature (about 23 °C). At 22% RH ambient and refrigerated storage samples showed no migration for acetoin or diacetyl. Recoveries are not corrected for extraction efficiency.

Table 4.5.3
Storage Test for Acetoin at 22% RH

time (days)	ambient storage recovery (%)			refrigerated storage recovery (%)		
0	100.2	99.8	97.9			
4	99.9	97.4	98.4	100.1	97.4	99.6
7	98.2	100.5	96.9	99.7	98.8	97.5
10	99.9	97.7	97.1	99.4	97.7	100.3
14	98.9	99.4	96.8	98.2	99.9	96.9
17	99.2	97.3	95.7	96.2	98.7	99.3

Table 4.5.4
Storage Test for Diacetyl at 22% RH

time (days)	ambient storage recovery (%)			refrigerated storage recovery (%)		
0	100.4	97.1	98.5			
4	99.9	98.2	97.0	99.5	100.1	97.3
7	99.6	98.8	97.1	99.9	98.7	97.4
10	99.9	98.1	96.9	99.8	98.9	97.0
14	99.7	96.5	98.4	99.5	98.0	96.8
17	99.0	98.0	95.7	98.1	99.3	96.3

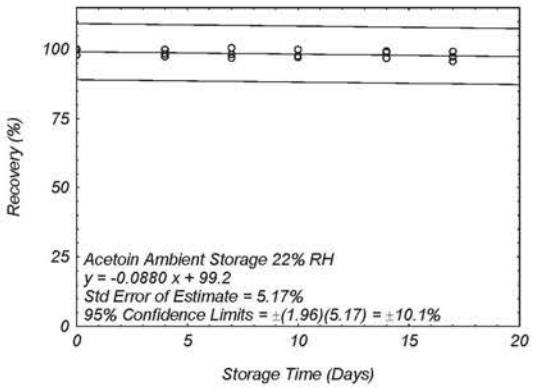

Figure 4.5.5. Ambient storage test for acetoin at 22% RH.

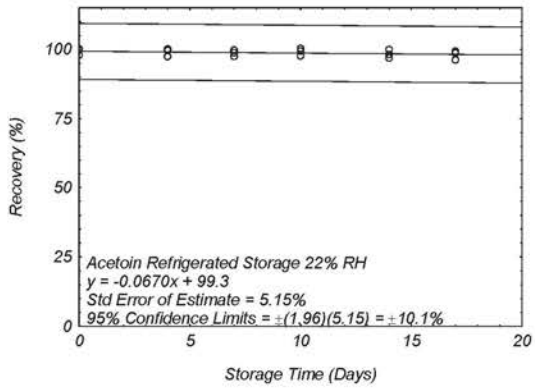

Figure 4.5.6. Refrigerated storage test for acetoin at 22% RH.

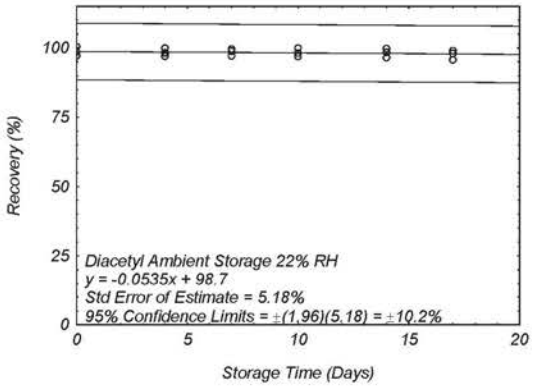

Figure 4.5.7. Ambient storage test for diacetyl at 22% RH.

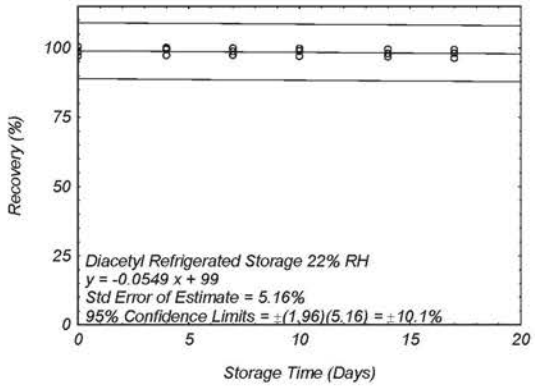

Figure 4.5.8. Refrigerated storage test for diacetyl at 22% RH.

At the beginning of this method, the SKC 226-183 tubes were available as a 400/200 mg tube. Migration studies showed that it would be necessary to use two tubes in series, so subsequent tubes were packed as a single 600 mg tube. A 600 mg section makes it easier for the analyst to prepare the samples for extraction. Migration occurs when the analyte equilibrates between the two sections of the tube after collection. There is more migration with higher humidities, due to the higher amounts of water collected. Using 400/200 mg dried silica gel tubes, at 80% RH acetoin showed no migration but the diacetyl refrigerated samples at day 18 showed a 4.5% migration and ambient showed 15.2% migration. Based on these results, a single 400/200 mg dried silica gel tube should not be used for sampling.

Table 4.5.5
Migration of Diacetyl on 400/200 mg Dried Silica Gel Tube
Sampled at 0.05 L/min for 180 min from 0.05 ppm Atmosphere

	ambient		refrigerated	
day	400 mg % of total found	200 mg % of total found	400 mg % of total found	200 mg % of total found
4	96.1	3.2	99.4	0.0
	96.0	4.1	100.1	0.0
	94.4	3.7	97.3	0.0
7	93.4	5.4	100.3	0.0
	92.9	5.8	99.3	0.0
	91.5	5.7	97.1	0.0
10	89.1	8.2	97.5	0.0
	91.3	8.5	100.0	0.0
	90.9	8.0	99.8	0.0
14	88.0	11.7	97.9	1.8
	87.8	11.3	97.3	1.6
	86.0	11.6	95.7	0.9
18	81.2	17.5	86.9	4.2
	82.7	15.0	85.5	4.5
	83.7	13.1	83.2	4.8

4.6 Reproducibility

Six samples were prepared from a controlled test atmosphere at the target concentration at an average relative humidity of 78% at 23 °C. The samples were submitted to the OSHA Salt Lake Technical Center for analysis, along with a draft copy of this method. The samples were analyzed after being stored at 4 °C for 20 days and at -12 °C for an additional 19 days. Sample results were corrected for extraction efficiency. No sample result for acetoin or diacetyl had a deviation greater than the precision of the overall procedure determined in Section 4.4.

Table 4.6.1
Reproducibility Data for Acetoin

theoretical (µg/sample)	recovered (µg/sample)	recovery (%)	deviation (%)
1.62	1.59	98.1	-1.9
1.65	1.53	92.7	-7.3
1.67	1.54	92.2	-7.8
1.66	1.56	94.0	-6.0
1.69	1.64	97.0	-3.0
1.64	1.51	92.1	-7.9

Table 4.6.2
Reproducibility Data for Diacetyl

theoretical (µg/sample)	recovered (µg/sample)	recovery (%)	deviation (%)
1.62	1.53	94.4	-5.6
1.64	1.48	90.2	-9.8
1.60	1.49	92.5	-7.5
1.61	1.50	93.2	-6.8
1.66	1.53	92.2	-7.8
1.62	1.50	92.6	-7.4

Samples that are prepared and analyzed by OSHA Method 1013[37] can be derivatized and re-analyzed by this method to detect lower levels. The following samples were prepared from a controlled test atmosphere at 0.51 ppm (0.184 mg/m^3) acetoin and 0.50 ppm (0.180 mg/m^3) diacetyl at 74% RH and 24 °C. They were submitted for analysis by OSHA Method 1013 and then reanalysis by OSHA Method 1012. The average acetoin recovery of samples analyzed by OSHA Method 1013 was 99.3% and by OSHA Method 1012 was 97.1%. The average diacetyl recovery of samples analyzed by OSHA Method 1013 was 98.9% and by OSHA Method 1012 was 96.6%.

[37] Simmons, M., Hendricks, W., Acetoin Diacetyl (OSHA Method 1013), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1013/1013.html (accessed 11/1/2008).

Table 4.6.3
Samples for Acetoin Analyzed by OSHA Method 1013 and Then by OSHA Method 1012

	OSHA Method 1013 GC-FID			OSHA Method 1012 GC-ECD		
theoretical (µg/sample)	recovered (µg/sample)	recovery (%)	deviation (%)	recovered (µg/sample)	recovery (%)	deviation (%)
16.5	16.4	99.4	-0.6	16.2	98.2	-1.8
16.4	16.2	98.8	-1.2	16.0	97.6	-2.4
16.6	16.3	98.2	-1.8	16.1	97.0	-3.0
15.9	16.1	101.3	+1.3	15.6	98.1	-1.9
16.5	16.1	97.6	-2.4	15.8	95.8	-4.2
16.3	16.4	100.6	+0.6	15.6	95.7	-4.3

Table 4.6.4
Samples for Diacetyl Analyzed by OSHA Method 1013 and Then by OSHA Method 1012

	OSHA Method 1013 GC-FID			OSHA Method 1012 GC-ECD		
theoretical (µg/sample)	recovered (µg/sample)	recovery (%)	deviation (%)	recovered (µg/sample)	recovery (%)	deviation (%)
16.0	15.9	99.4	-0.6	15.6	97.5	-1.8
15.7	15.4	98.1	-1.9	15.1	96.2	-2.4
15.8	15.5	98.1	-1.9	15.1	95.6	-3.0
15.6	15.8	101.3	+1.3	15.2	97.4	-1.9
15.7	15.2	96.8	-3.2	15.0	95.5	-4.2
15.9	15.8	99.4	-0.6	15.5	97.5	-4.3

4.7 Sampler capacity

The sampling capacity of the front tube of two dried silica gel tubes in series was tested by sampling from a dynamically generated test atmosphere with an average relative humidity of 81% at 23°C at concentrations of 0.101 ppm (0.365 mg/m^3) acetoin, and 0.101 ppm (0.355 mg/m^3) diacetyl. The second tube in the sampling train was changed at 1 h intervals for the first 3 hours then at 0.5 hour intervals for the rest of the sampling. The dried silica gel tube sampling trains were used to sample at approximately 0.05 L/min (each air volume listed below uses that specific tube's flow rate). The presence of analyte on the second tube was defined as breakthrough. The percentage of the amount found on the second tube of the total concentration is the % breakthrough. The % breakthrough was plotted versus the air volume sampled to determine the 5% breakthrough air volumes. The 5% breakthrough air volume for diacetyl was 12.1 L. The recommended air volume is 80% of the breakthrough air volume which is 9.68 L. Acetoin had no breakthrough after samples were collected for up to 8 hours.

Table 4.7.1
Capacity Test for Diacetyl on Dried Silica Gel Tubes at 0.101 ppm

sampling train 1		sampling train 2		sampling train 3	
air volume	% BT	air volume	% BT	air volume	% BT
2.71	0.0	2.80	0.0	2.78	0.0
5.51	0.0	5.69	0.0	5.67	0.0
8.36	0.0	8.64	0.0	8.60	0.0
9.69	0.0	10.0	0.0	9.97	0.0
12.0	5.2	12.6	27.8	12.5	20.3

%BT = % breakthrough

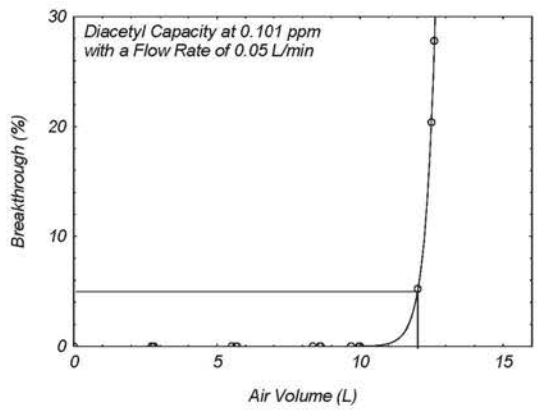

Figure 4.7.1. Five percent breakthrough test for diacetyl from a 0.101 ppm atmosphere, with a flow rate of 0.05 L/min.

A capability of collection at higher flow rates with a 15 minute short term sample was tested for breakthrough. A test atmosphere was dynamically generated with an average relative humidity of 79% at 23 °C at concentrations of 0.101 ppm (0.365 µg/m^3) acetoin and 0.101 ppm (0.355 mg/m^3) diacetyl. A sampling train consisting of two dried silica gel tubes (400/200 mg) in series was used to test the capacity. Three sampling trains at each flow rate of 0.1 L/min or 0.2 L/min were tested. There was no acetoin or diacetyl on the second tube of any of the sampling trains. Since the short term sampling may be a time of higher exposure, two higher concentrations were also tested. The first was 0.541 ppm (1.95 mg/m^3) acetoin and 0.506 ppm (1.78 mg/m^3) diacetyl and a relative humidity of 79% at 23 °C. The second was 23.2 ppm (83.5 mg/m^3) acetoin and 22.4 ppm (78.8 mg/m^3) diacetyl at an average relative humidity of 79% at 23 °C. In all of these tests there was no acetoin or diacetyl on the back-up tube of the sampling train.

Table 4.7.2
15 min Capability to Sample at 0.2 L/min from an Atmosphere of 0.101 ppm Acetoin and 0.101 ppm Diacetyl

	acetoin		diacetyl	
flow rate (L/min)	front tube (%)	back tube (%)	front tube (%)	back tube (%)
0.1	98.6	0.0	99.4	0.0
0.1	99.4	0.0	98.7	0.0
0.1	99.9	0.0	99.1	0.0
0.2	99.2	0.0	99.5	0.0
0.2	98.5	0.0	98.4	0.0
0.2	97.7	0.0	99.8	0.0

Table 4.7.3
15 min Capability to Sample at 0.2 L/min from an Atmosphere of 0.541 ppm Acetoin and 0.506 ppm Diacetyl

	acetoin		diacetyl	
flow rate (L/min)	front tube (%)	back tube (%)	front tube (%)	back tube (%)
0.1	99.7	0.0	99.9	0.0
0.1	99.0	0.0	98.4	0.0
0.1	98.8	0.0	97.9	0.0
0.2	99.3	0.0	99.4	0.0
0.2	97.9	0.0	98.9	0.0
0.2	99.5	0.0	99.0	0.0

Table 4.7.4
15 min Capability to Sample at 0.2 L/min from an Atmosphere of 23.2 ppm Acetoin and 22.4 ppm Diacetyl

	acetoin		diacetyl	
flow rate (L/min)	front tube (%)	back tube (%)	front tube (%)	back tube (%)
0.1	98.6	0.0	99.6	0.0
0.1	99.4	0.0	98.7	0.0
0.1	99.0	0.0	97.3	0.0
0.2	99.9	0.0	99.6	0.0
0.2	97.5	0.0	99.0	0.0
0.2	98.1	0.0	97.8	0.0

A capacity test at 0.2 L/min was performed at two test air concentrations, 0.101 ppm (0.365 mg/m^3) acetoin and 0.101 ppm (0.355 mg/m^3) diacetyl at an average relative humidity of 78% air at 22 °C; and 23.2 ppm (83.5 mg/m^3) acetoin and 22.4 ppm (78.8 mg/m^3) diacetyl at relative humidity of 77% at 22 °C. There was no acetoin on the back-up tube after 13.9 L was sampled. The 5% breakthrough air volume for diacetyl with 0.101 ppm atmosphere was 11.98 L, and with a 22.4 ppm atmosphere was 11.64 L.

Table 4.7.5
Capacity Test for Diacetyl on Dried Silica Gel Tubes at a Flow Rate of 0.2 L/min and 0.101 ppm

sampling train 1		sampling train 2		sampling train 3	
air volume	% BT	air volume	% BT	air volume	% BT
5.98	0.0	5.95	0.0	6.03	0.0
7.97	0.0	7.94	0.0	8.04	0.0
9.97	0.0	9.92	0.0	10.05	0.0
10.96	0.7	10.91	0.0	11.06	1.4
11.96	5.4	11.90	3.4	12.06	8.8
12.95	26.4	12.90	22.7	13.07	35.1

%BT = % breakthrough

Table 4.7.6
Capacity Test for Diacetyl on Dried Silica Gel Tubes
at a Flow Rate of 0.2 L/min and 22.4 ppm

sampling train 1		sampling train 2		sampling train 3	
air volume	% BT	air volume	% BT	air volume	% BT
6.15	0.0	5.94	0.0	6.06	0.0
8.20	0.0	7.92	0.0	8.08	0.0
10.25	0.0	9.90	0.0	10.10	0.0
11.28	2.1	10.89	0.6	11.11	1.2
12.30	17.2	11.88	5.1	12.12	10.5
13.33	48.5	12.87	24.1	13.13	40.5

%BT = % breakthrough

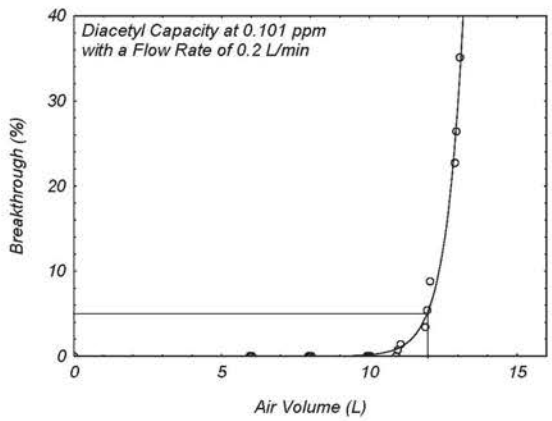

Figure 4.7.2. Five percent breakthrough test for diacetyl from a 0.101 ppm atmosphere, with a flow rate of 0.2 L/min.

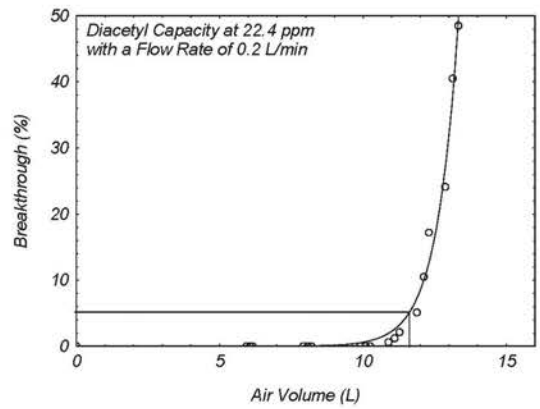

Figure 4.7.3. Five percent breakthrough test for diacetyl from a 22.4 ppm atmosphere, with a flow rate of 0.2 L/min.

4.8 Extraction efficiency and stability of extracted samples

The extraction efficiency is dependent on the extraction solvent as well as the internal standard. The extraction solvent used for this evaluation consisted of 95:5 ethyl alcohol:water with 2 mg/mL PFBHA and 20 µg/mL 4-bromobenzyl bromide. Other extraction solvents or internal standards may be used provided that the new extraction solution or internal standard is tested. The new extraction solvent or internal standard should be tested as described below.

Extraction efficiency

The extraction efficiencies of acetoin and diacetyl were determined by liquid-spiking four dried silica gel tubes, at each concentration level, with the analyte from the RQL to 2 times the target concentration. These samples were stored overnight at ambient temperature and then analyzed. The samples need to be extracted on a rotator for 1 hour, and then allowed to set at room temperature for 36 hours. Do not use a shaker as recoveries will be much lower (Table 4.8.3). The mean extraction efficiency over the working range from the RQL to 2 times the target concentration is 102.0% for acetoin and 97.6% for diacetyl. The extraction efficiency for the wet samplers and samplers extracted on the shaker were not included in the overall mean because it would bias the results. The test of wet samplers was performed to determine if the amount of water that would collect under high humidity conditions at the recommended air volume would affect the extraction efficiency. Wet samplers were prepared by sampling humid

air having an average relative humidity of about 80% at 23 °C for 180 minutes at 0.05 L/min and then liquid-spiking the sampler with the analyte. The dried silica gel tube (600 mg) collects 140 mg water at 78% RH and 23 °C when sampled for 9 L.

Table 4.8.1
Extraction Efficiency (%) of Acetoin

level		sample number				mean
× target concn	µg per sample	1	2	3	4	
RQL	0.022	104.2	102.1	101.2	101.6	102.3
0.25	0.41	103.7	102.3	102.1	100.8	102.2
0.5	0.82	100.7	102.4	101.1	100.9	101.3
1.0	1.64	102.3	100.5	103.3	103.5	102.4
1.5	2.46	102.6	103.1	100.6	100.8	101.8
2.0	3.28	103.0	103.3	101.6	100.4	102.1
1.0 (wet)	1.64	101.1	102.9	103.1	102.2	102.3

Table 4.8.2
Extraction Efficiency (%) of Diacetyl

level		sample number				mean
× target concn	µg per sample	1	2	3	4	
RQL	0.02	96.7	95.7	97.8	98.9	97.3
0.25	0.40	97.5	98.0	99.1	98.5	98.3
0.5	0.79	98.5	96.8	99.4	98.0	98.2
1.0	1.58	96.9	95.3	96.4	95.4	96.0
1.5	2.37	99.9	95.9	96.5	97.8	97.5
2.0	3.16	97.1	99.6	99.9	97.5	98.5
1.0 (wet)	1.58	98.1	96.6	95.8	97.1	96.9

Table 4.8.3
Extraction Efficiency (%) of Acetoin and Diacetyl at 1.0 x Target Concentration Using a Shaker

		sample number				
analyte	µg per sample	1	2	3	4	mean
acetoin	1.64	87.5	88.8	90.1	87.7	88.5
diacetyl	1.58	82.6	81.9	85.5	84.3	83.6

Stability of extracted samples

The stability of extracted samples was investigated by reanalyzing the target concentration samples 24 h after initial analysis. After the original analysis was performed, two autosampler vials were recapped with new septa while the remaining two retained their punctured septa. The samples were reanalyzed with fresh standards. The average percent change was +0.7% for acetoin and +1.6% for diacetyl when samples were resealed with new septa and -1.1% for acetoin and +0.3% for diacetyl when samples retained their punctured septa. Each septum was punctured 5 times for each analysis. The test was performed at room temperature.

Table 4.8.4
Stability of Extracted Samples for Acetoin

punctured septa replaced			punctured septa retained		
initial (%)	after one day (%)	difference (%)	initial (%)	after one day (%)	difference (%)
102.3	101.5	-0.8	103.3	101.9	-1.4
100.5	102.7	+2.2	103.5	102.7	-0.8
	(mean)			(mean)	
101.4	102.1	+0.7	103.4	102.3	-1.1

Table 4.8.5
Stability of Extracted Samples for Diacetyl

punctured septa replaced			punctured septa retained		
initial (%)	after one day (%)	difference (%)	initial (%)	after one day (%)	difference (%)
96.9	98.3	+1.4	96.4	95.1	-1.3
95.3	97.1	+1.8	95.4	97.3	+1.9
	(mean)			(mean)	
96.1	97.7	+1.6	95.9	96.2	+0.3

4.9 Interferences (sampling)

Retention

The ability of a dried silica gel tube to retain the analytes after they have been collected was tested by using a test atmosphere having an average relative humidity of 80% at 23 °C. The test atmosphere was dynamically generated at 0.101 ppm (0.364 mg/m^3) acetoin, and 0.102 ppm (0.359 mg/m^3) diacetyl. Six samplers had contaminated air drawn through them at 0.05 L/min for 45 min. Sampling was discontinued and three samples set aside. The generation system was flushed with contaminant-free air. Sampling resumed with the other three samples having contaminant-free air drawn through them at 0.05 L/min for 135 min and then all six samplers were analyzed. The mean recoveries for the samples in the second set divided by the first set were: 96.7% for acetoin, and 96.9% for diacetyl.

Table 4.9.1
Retention of Acetoin

	percent recovery			
set	1	2	3	mean
first	99.5	100.4	98.9	99.6
second	95.0	96.8	97.0	96.3
second/first				96.7

Table 4.9.2
Retention of Diacetyl

	percent recovery			
set	1	2	3	mean
first	100.2	99.9	98.1	99.4
second	96.3	97.4	95.3	96.3
second/first				96.9

Low humidity

The ability of dried silica gel tubes to collect the analytes from a relatively dry atmosphere was tested by using a test atmosphere having an average relative humidity of 20% at 23 °C. The test atmosphere was dynamically generated at 0.101 ppm (0.364 mg/m^3) acetoin and 0.102 ppm (0.359 mg/m^3) diacetyl. Three samplers had contaminated air drawn through them at 0.05 L/min for 180 min. All of the samples were immediately analyzed. The recoveries (% of theoretical) for acetoin were: 97.0%, 101.4%, and 97.8%; and for diacetyl were: 98.3%, 96.8%, and 100.3%.

Low concentration

The ability of dried silica gel tubes to collect the analytes from a low concentration atmosphere was tested by using a test atmosphere at 0.1 times the target concentration having an average relative humidity of 80% at 23 °C. The test atmosphere was dynamically generated at 0.0051 ppm (0.0184 mg/m^3) acetoin and 0.0051 ppm (0.0180 mg/m^3) diacetyl. Three samplers had contaminated air drawn through them at 0.05 L/min for 180 min. All of the samples were immediately analyzed. The recoveries (% of theoretical) for acetoin were: 99.8%, 99.9%, and 97.2%, and for diacetyl were: 97.3%, 98.1%, and 99.8%.

Sampling interference

The ability of dried silica gel tubes to collect the analytes from an atmosphere containing interferences was tested under two different sets of conditions. The first set of conditions was a test atmosphere of 0.051 ppm (0.0184 mg/m^3) acetoin and 0.051 ppm (0.0180 mg/m^3) diacetyl and an interference mixture of 1.01 ppm (1.82 mg/m^3) acetaldehyde, 1.05 ppm (2.58 mg/m^3) acetic acid, and 1.02 ppm (3.01 mg/m^3) methyl ethyl ketone at an average humidity of 80% at 23 °C. These lower concentrations were chosen for two reasons: they are similar to some of the concentrations found in plants manufacturing microwave popcorn, and all of these compounds will be derivatized by the PFBHA; therefore, there would be enough PFBHA in solution to derivatize all of the analytes that were collected (8.01 µmole/mL PFBHA). The recoveries (% of theoretical) of acetoin and diacetyl were: 95.4%, 98.5%, and 99.7% for acetoin and 95.8%, 98.9%, and 99.8% for diacetyl. There was no analyte on the backup tube of the two dried silica gel tubes in series for any of the tests.

The second series of tests was with acetoin and diacetyl at the target concentration and each of the interferences listed above individually at their PEL concentration following the guidelines in SLTC "Evaluation Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis"[38]. The concentrations of these interferences are much higher than would normally be expected in a food or flavoring manufacturing workplace. These three compounds were chosen as interferences because they collect on the dried silica gel tubes and react with the PFBHA. The extraction solution needed to be modified to 18 mg/mL PFBHA (72.1 µmoles/mL) to insure that there was enough PFBHA in solution to derivatize all the analytes. These three atmospheres each contained acetoin and diacetyl with one of the following concentrations of the interference mixture in it: 194 ppm (350 mg/m^3) acetaldehyde, 9.49 ppm (23.3 mg/m^3) acetic acid, or 190 ppm (560 mg/m^3) methyl ethyl ketone. Three samplers had contaminated air drawn through them at 0.05 L/min for 180 min for each test. All of the samples were immediately analyzed. The recoveries (% of theoretical) of acetoin and diacetyl with 190 ppm acetaldehyde were: 99.8%, 95.9%, and 97.7% for acetoin and 97.2%, 93.5%, and 95.7% for diacetyl. The recoveries (% of theoretical) of acetoin and diacetyl with 9.49 ppm acetic acid were: 95.3%, 97.7%, and 98.9% for acetoin and 95.5%, 99.3%, and 99.8% for diacetyl. The recoveries (% of theoretical) of acetoin and diacetyl with 190 ppm methyl ethyl ketone were: 96.7%, 98.7%, and 99.9% for acetoin and 95.8%, 97.8%, and 99.3% for diacetyl. There was no analyte found on the backup tube of the two dried silica gel tubes in series for any of the tests. These interferences were not a sampling interference, but under normal sample analysis, these levels of interferences would be an analytical interference.

Light

Diacetyl and acetoin are light-sensitive.[39,40,41,42] The interference of light during sampling was tested using three foil-wrapped sampling trains and three uncovered sampling trains. An

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38 *Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis, 1999. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/chromguide/index.html (accessed 3/15/2008).*

39 *Material Safety Data Sheet: Acetoin, http://www.thegoodscentscompany.com/msds/md102388.html (accessed 3/17/2008).*

atmosphere containing twice the target concentration at an average humidity of 78% at 23 °C was sampled for 180 min at 0.05 L/min, and the samples were extracted that day.

Table 4.9.3
Light Interference During Sampling

	acetoin		diacetyl	
tube #	foil wrapped recovery (%)	uncovered recovery (%)	foil wrapped recovery (%)	uncovered recovery (%)
1	98.9	93.7	97.8	93.3
2	97.0	92.6	98.9	94.6
3	99.5	95.4	99.9	95.0
mean	98.5	93.9	98.9	94.3

An additional three sampling trains were collected at the same time, and were protected from the light by aluminum foil. After collection, these samplers had the foil removed and were placed on the counter at ambient temperature under room light. These samples were analyzed 24 h after sampling during which they were exposed to the room light for 14 of the 24 h, and the recoveries were 80.7%, 84.7%, and 78.5% for acetoin and 79.3%, 82.4%, and 78.4% for diacetyl.

Powder form

The powder form of acetoin and diacetyl tested consisted of starch coated with acetoin and diacetyl. Three tests were performed on this powder. The first consisted of a sampling train of a pre-weighed (tared) PVC filter in a conical cassette in series with two dried silica gel tubes. Two dried silica gel tubes were used to collect any vapors of acetoin and diacetyl which would be stripped off of the powder. Known amounts of the powder were placed onto the PVC filter, and 9 L of air at an average relative humidity of 78% RH and 22 °C were pulled through the sampling trains at 0.05 L/min. The recovery of acetoin and diacetyl on the pre-weighed PVC filters was 0% to 1.9% for acetoin and 0% to 2.3% for diacetyl, with larger amounts found on the PVC filters that were spiked with larger amounts of powder. Most of the acetoin and diacetyl was stripped from the starch and collected on the dried silica gel tubes. The average recovery found on the dried silica gel tubes was 96.6% for acetoin and 97.8% for diacetyl (Table 4.9.4). The acetoin and diacetyl theoretical weights were calculated from the percentages obtained from analysis of the powder and the amounts of the powder weighed out.

The second and third tests consisted of a sampling train of two dried silica gel tubes in series, with the powder spiked on the front glass wool of the front tube. The two tests had 9 L air drawn through the sampling trains at 0.05 L/min, the first test used air at an average relative humidity of 20% at 22 °C, and the other test used air at an average relative humidity of 78% at 22 °C. At 20% RH most of the acetoin and diacetyl were found on the front glass wool and glass fiber filter, but at 78% RH most of the acetoin and diacetyl were found on the dried silica gel beds. The sampling trains with 78% RH air drawn through them had the highest amounts of acetoin and diacetyl on the glass wool and filter on the tube spiked with the highest amount of powder, which may be due to the size of the clump of powder weighed out (Table 4.9.5 and 4.9.6).

[40] *Material Safety Data Sheet: Diacetyl, Chemwatch, Victoria, Australia (accesed 3/17/2008).*
[41] *Material Safety Data Sheet: 2,3-Butanedione, https://fscimage.fishersci.com/msds/03275.htm (accessed 3/17/2008).*
[42] *Material Safety Data Sheet: 2,3-Butanedione, http://www.chemservice.com/msds/msds_detail.asp?catnum=O-816 (accessed 3/17/2008).*

Table 4.9.4
% Recovery of Acetoin and Diacetyl from Powder on Tared PVC Filters in a Conical Cassette in Series with Dried Silica Gel Tubes with 78% RH Air Sampled

		acetoin					diacetyl				
amount of powder (µg)	powder weight found (µg)	theoretical weight (µg)	PVC filter (µg)	front tube (µg)	back tube (µg)	silica gel recovery (%)	theoretical weight (µg)	PVC filter (µg)	front tube (µg)	back tube (µg)	silica gel recovery (%)
1130	1082	18.1	0.0	18.0	0.0	99.4	29.4	0.0	28.0	0.0	95.2
2110	2021	33.8	0.6	32.1	0.0	95.0	54.9	1.0	53.1	0.0	96.7
2960	2856	47.4	0.9	46.3	0.0	97.7	77.0	1.8	75.9	0.0	98.6
2940	2809	47.0	0.3	45.0	0.0	95.7	76.4	0.8	75.7	0.0	99.1
1310	1265	21.0	0.2	20.5	0.0	97.6	34.1	0.6	34.0	0.0	99.7
1010	964	16.2	0.0	15.3	0.0	94.4	26.3	0.0	25.6	0.0	97.3

Table 4.9.5
% Recovery of Acetoin and Diacetyl from Powder Spiked on Dried Silica Gel Tubes with 20% RH Air Sampled

		acetoin					diacetyl					
amount of powder (µg)	theoretical weight (µg)	front glass wool and filter (µg)	front glass wool and filter recovery (%)	front tube (µg)	back tube (µg)	silica gel recovery (%)	theoretical weight (µg)	front glass wool and filter (µg)	front glass wool and filter recovery (%)	front tube (µg)	back tube (µg)	silica gel recovery (%)
1080	17.3	16.7	96.5	0.0	0.0	0.0	28.1	26.3	93.6	1.1	0.0	3.9
1240	19.8	19.5	98.5	0.0	0.0	0.0	32.2	30.1	93.5	1.5	0.0	4.7
1750	28.0	27.4	97.9	0.0	0.0	0.0	45.5	42.8	94.1	1.8	0.0	4.0
2080	33.3	32.1	96.4	0.0	0.0	0.0	54.1	50.2	92.8	2.3	0.0	4.3
2240	35.8	34.5	96.4	0.5	0.0	1.4	58.2	53.4	91.8	2.8	0.0	4.8
2380	38.1	36.7	96.3	0.7	0.0	1.8	61.9	55.8	90.1	3.6	0.0	5.8

Table 4.9.6
% Recovery of Acetoin and Diacetyl from Powder Spiked on Dried Silica Gel Tubes with 78% RH Air Sampled

		acetoin					diacetyl					
amount of powder (µg)	theoretical weight (µg)	front glass wool and filter (µg)	front glass wool and filter recovery (%)	front tube (µg)	back tube (µg)	silica gel recovery (%)	theoretical weight (µg)	front glass wool and filter (µg)	front glass wool and filter recovery (%)	front tube (µg)	back tube (µg)	silica gel recovery (%)
1220	19.5	0.0	0.0	19.1	0.0	97.9	31.7	0.0	0.0	30.9	0.0	97.5
1760	28.2	0.0	0.0	26.9	0.0	95.4	45.8	0.0	0.0	44.2	0.0	96.5
1070	17.1	0.0	0.0	16.9	0.0	98.8	27.8	0.0	0.0	27.5	0.0	98.9
1590	25.4	0.0	0.0	24.9	0.0	98.0	41.3	0.0	0.0	40.9	0.0	99.0
2030	32.5	0.0	0.0	32.4	0.0	99.7	52.8	0.0	0.0	52.5	0.0	99.4
5020	80.3	0.7	0.9	79.4	0.0	98.9	130.5	2.2	1.7	129.9	0.0	99.5

4.10 Qualitative analysis

When necessary, the identity or purity of an analyte peak can be confirmed by GC-mass spectrometry or by another analytical procedure. The mass spectra of the acetoin-PFBHA and diacetyl-PFBHA derivative were determined by analyzing an analytical standard on an Agilent 6890 with a 5973 mass selective detector using a 30-m × 0.25-mm i.d. fused silica capillary column (DB-1-MS 0.25-µm df) capillary column at a temperature program of 50 °C, hold 2 min, program at 10 °C/min up to 180 °C hold 10 min, with injection port at 240 °C and mass spectrometer at 250 °C.

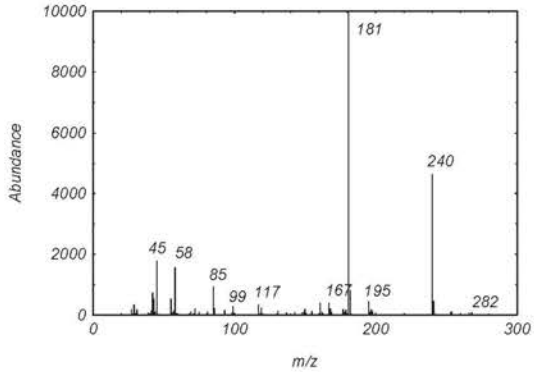

Figure 4.10.1. Mass spectrum of acetoin-PFBHA derivative.

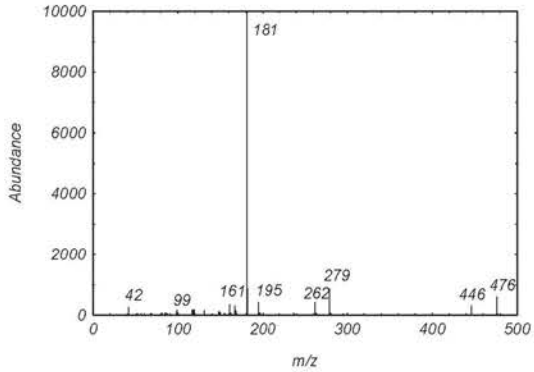

Figure 4.10.2. Mass spectrum of diacetyl-PFBHA derivative.

4.11 Generation of test atmospheres

The test atmosphere of acetoin and diacetyl was generated from a water solution.

The following apparatus was placed in a walk-in hood. The acetoin and diacetyl vapors were generated by pumping the solution, using the Isco pump, through a short length of 0.53-mm uncoated fused silica capillary tubing into a vapor generator where it was heated and evaporated into the dilution air stream (Figure 4.11). The vapor generator consisted of a 15-cm length of 5-cm diameter glass tubing with a side port for introduction of the capillary tubing. The glass tube of the vapor generator was wrapped with heating tape to evaporate the chemicals. The humidity, temperature, and volume of the dilution stream of air were regulated by use of a Miller Nelson Flow-Temperature-Humidity controller. The test atmosphere passed into a glass mixing chamber (76-cm × 30-cm) from the vapor generator, and then into a glass exposure chamber (76-cm × 20-cm). Active samplers were attached to glass tubes extending from the exposure chamber. The humidity and temperature were measured at the exit of the exposure chamber with an Omega Digital Thermo-hygrometer.

Generation of test atmospheres required extra heating of the air stream to vaporize the acetoin. The temperature and humidity were measured after the air had exited the sampling chamber. The air stream cooled as it passed from the mixing chamber to the sampling chamber and then out the exit. While the air coming out of the exit was 23 °C and 80% RH, the temperature measured in the front of the sampling chamber was 30 °C and 54% RH, giving similar absolute humidities of 16.4 mg/L H_2O.

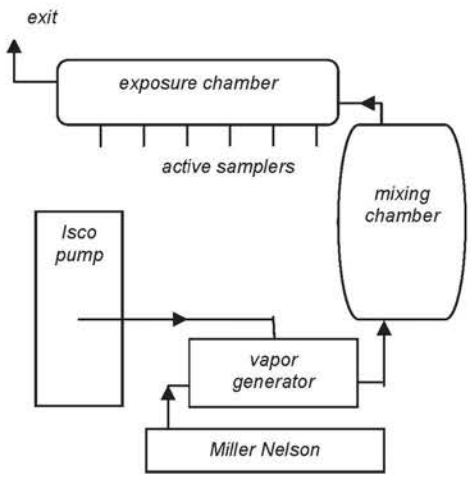

Figure 4.11. The test atmosphere generation and sampling apparatus.

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Appendix C

Acetoin Diacetyl 1013

Acetoin
Diacetyl

Method no.:	1013
Control no.:	T-1013-FV-01-0809-M
Target concentration:	0.5 ppm (1.80 mg/m^3) acetoin 0.5 ppm (1.76 mg/m^3) diacetyl
OSHA PEL:	none for acetoin none for diacetyl
ACGIH TLV:	none for acetoin none for diacetyl
Procedure:	Samples are collected by drawing workplace air through two sampling tubes, containing specially dried and cleaned silica gel, connected in series. Samples are extracted with ethyl alcohol:water (95:5) and analyzed by gas chromatography (GC) using a flame ionization detector (FID).
Recommended sampling time and sampling rate:	180 min at 0.05 L/min (9 L) (TWA) 15 min at 0.2 L/min (3 L) (short term)
Reliable quantitation limit:	0.011 ppm (0.039 mg/m^3) acetoin 0.012 ppm (0.041 mg/m^3) diacetyl
Standard error of estimate at the target concentration:	5.7% acetoin 5.2% diacetyl
Special requirement:	Protect samples from light exposure during sampling, shipping and analysis.
Status of method:	Evaluated method. This method has been subjected to the established evaluation procedures of the Methods Development Team.

September 2008

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1. General Discussion

For assistance with accessibility problems in using figures and illustrations presented in this method, please contact the Salt Lake Technical Center (SLTC) at (801) 233-4900. This procedure was designed and tested for internal use by OSHA personnel. Mention of any company name or commercial product does not constitute endorsement by OSHA.

1.1 Background

1.1.1 History

In 2003 OSHA issued Method PV2118[1] for sampling and analysis of diacetyl using two silica gel sorbent tubes (150/75 mg) in series. PV2118 has a recommended sampling volume of 3 L and a reliable quantitation limit of 3 µg (0.28 ppm). In 2003 NIOSH issued Method 2557[2] for diacetyl and Method 2558[3] for acetoin. Both methods use Anasorb CMS sorbent (150/75 mg) tubes, can sample up to 10 L of air and have a limit of detection for acetoin of 1 µg and 0.6 µg for diacetyl. These two methods use slightly different acetone/methanol extraction solvents and were not optimized for simultaneous analysis of both analytes. In 2008 a note was placed on NIOSH Method 2557 indicating that high humidity is a sampling interference that results in underestimation of the true concentration.

In September of 2007, OSHA published a Hazard Communication Guidance Document[4] and a Safety and Health Information Bulletin on Respiratory Disease among Employees in Microwave Popcorn Processing Plants[5] for diacetyl. Due to the increasing concern of workplace exposure to diacetyl, two new sampling and analytical methods were validated that permitted longer sampling times and had lower quantitation limits than PV2118. The new methods were also validated for acetoin because it has been found in facilities in which diacetyl was in use.

This procedure, Method 1013, was streamlined for monitoring low ppm levels, and Method 1012[6] was optimized for ppb levels. Both methods use two 600 mg silica gel sorbent tubes in series. Both methods have a recommended sampling time of 3 hours (9 L) and both use the same solvent for sample extraction. However, in Method 1012, acetoin and diacetyl are derivatized using *O*-pentafluorobenzyl hydroxylamine hydrochloride. This derivatization results in a reliable quantitation limit approximately 10 times less than Method 1013. The disadvantage of derivatizing acetoin and diacetyl is that the derivatization step requires 36 hours; whereas, with this method sample preparation can be performed in 1 hour. Also, samples extracted and analyzed according to this procedure can then be derivatized and analyzed using Method 1012, if needed.

The silica gel used in the sampler for this method, and for Method 1012, has been specially cleaned and dried as described in Appendix A. It was found that sampler

[1] Shah, Y. C. Diacetyl (OSHA Method PV2118), 2003. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/partial/t-pv2118/t-pv2118.html (accessed July 2008).
[2] Pendergrass, S. M. Diacetyl (NIOSH Method 2557), 2003. Centers for Disease Control and Prevention, National Institute of Occupational Safety and Health Web site. http://www.cdc.gov/niosh/nmam/pdfs/2557.pdf (accessed July 2008).
[3] Pendergrass, S. M. Acetoin (NIOSH Method 2558), 2003. Centers for Disease Control and Prevention, National Institute of Occupational Safety and Health Web Site. http://www.cdc.gov/niosh/nmam/pdfs/2558.pdf (accessed July 2008).
[4] Hazard Communication Guidance for Diacetyl and Food Flavorings Containing Diacetyl, 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dsg/guidance/diacetyl-guidance.html (accessed July 2008).
[5] Respiratory Disease Among Employees in Microwave Popcorn Processing Plants, 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/shib/shib092107.html (accessed July 2008).
[6] Eide, M. Acetoin and Diacetyl (OSHA Method 1012), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1012/1012.html (accessed September 2008).

capacity for diacetyl was not based on analyte concentration but limited by the amount of water remaining on the silica gel after cleanup and on the amount of water collected during sampling. In other words, the silica gel tube acts as a chromatography column and water elutes the collected diacetyl. By removing as much water as possible from the silica gel prior to sampling, the sampling volume for diacetyl can be increased because the time required to saturate the silica gel during sampling increases. Diacetyl was also found to gradually migrate within the sampling tube during storage resulting in the need to use a second tube in series during sampling in order to detect breakthrough. Acetoin has no capacity or migration issues on silica gel at the recommended sampling volume.

The powder and liquid formulated forms of acetoin and diacetyl may contain oily compounds and other base materials such as maltodrextin. These materials could affect the extraction of acetoin and diacetyl from the silica gel. The sampler contains a front glass wool plug followed by a glass fiber filter that serves only to trap any of these materials before they enter the silica gel bed. Retention studies using a powder containing acetoin and diacetyl showed the acetoin and diacetyl can be stripped off the powder and collected on the silica gel. These studies demonstrate that the glass fiber filter is not an efficient collector for diacetyl and acetoin, and will not normally be analyzed (see OSHA Method 1012[7], Section 4.9).

1.1.2 Toxic effects (This section is for information only and should not be taken as the basis of OSHA policy.)

Exposure to acetoin may result in skin, eyes, nose and throat irritation.[8]

Exposure to diacetyl "liquid or vapors can cause irritation to the skin, eyes, nose, and throat". "Animals exposed to diacetyl experienced damage to the nose and upper airways, including severe damage to cells lining the respiratory tract" and "NIOSH has reported that employees exposed to butter flavorings containing diacetyl are at risk of developing occupational lung diseases".[9]

Diacetyl, and to some extent acetoin, may be responsible for the occurrence of a rare and potentially fatal lung disease, bronchiolitis obliterans, among workers in microwave popcorn manufacturing plants and flavor manufacturing plants.[10] Symptoms of bronchiolitis obliterans include cough, shortness of breath with exertion, and spirometry test results showing fixed airways obstruction.[11]

Acetoin and diacetyl are used in the production of powdered flavorings.[12] These powdered flavorings may provide a means to deliver the substances deep into the lungs of exposed workers, however, the significance of this form of exposure is presently unknown.[13]

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[7] Eide, M. Acetoin and Diacetyl (OSHA Method 1012), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1012/1012.html (accessed September 2008).

[8] Acetyl Methyl Carbinol (Chemical Sampling Information), 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/chemicalsampling/data/CH_217010.html (accessed July 2008).

[9] Hazard Communication Guidance for Diacetyl and Food Flavorings Containing Diacetyl, 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dsg/guidance/diacetyl-guidance.html (accessed July 2008).

[10] van Rooy, F.; et al. Bronchiolitis Obliterans Syndrome in Chemical Workers Producing Diacetyl for Food Flavoring. *Am. J. Crit. Care Med.* **2007**, 176 (5), 498-504.

[11] Kanwal, R. Bronchiolitis obliterans in workers exposed to flavoring chemicals. *Curr Opin Pulm Med.* **2008**, 14 (2), 141-6.

[12] Kanwal, R.; Kullman, G. Report on Severe Fixed Obstructive Lung Disease in Workers at a Flavoring Manufacturing Plant Health Hazard Evaluation Report #2006-0303-3043, 2007. Centers for Disease Control and Prevention, National Institute of Occupational Safety and Health Web site. http://www.cdc.gov/niosh/hhe/reports/pdfs/2006-0303-3043.pdf (accessed July 2008) pp 11-13.

[13] Boylstein, R. J.; et al. Diacetyl Emissions and Airborne Dust from Butter Flavorings Used in Microwave Popcorn Production. *J. Occup. Environ. Hyg.* **2006**, 3 (10), 530-535.

1.1.3 Workplace exposure

Acetoin has a somewhat creamy taste and a woody yogurt odor. It is used as an ingredient in yogurt, butter, milk and strawberry flavors. It occurs naturally in foods such as wines, chesses, fruits and vegetables.[14] Occupational exposures can occur by inhalation or skin contact in locations where it is produced, used as a food additive, or used to produce flavorings or aromas.

Diacetyl has a strong butter odor in dilute form and a chlorine-quinone odor when concentrated. It is used as an ingredient to produce a butter flavor in many foods and beverages. It occurs naturally in alcoholic and nonalcoholic beverages, dairy products, fruits, plants, vegetables, meats, and natural aromas.[15] Like acetoin, occupational exposures to diacetyl can occur by inhalation or skin contact in locations where it is produced, used as a food additive, or used to produce flavorings or aromas.

Recently, occupational exposure to butter flavorings in the production of microwave popcorn and in other industries has received much publicity. NIOSH has identified acetoin and diacetyl as useful indicator compounds that can be used to represent exposure to butter flavorings.[16] Areas of special concern include flavor production rooms, areas where mixing/blending operations occur, packing/packaging operations, areas where flavors are handled openly, rooms where mixing tanks are located, quality control laboratories, and maintenance and cleaning operations.[17, 18]

1.1.4 Physical properties and other descriptive information

<u>Acetoin</u>[19, 20]

Acetoin occurs as the liquid monomer and the solid dimer. The monomer can be formed from the dimer by dissolving in water or other solvents.

synonyms:	acetyl methyl carbinol; 2,3-butanolone; dimethylketol; γ-hydroxy-β-oxobutane; 1-hydroxyethyl methyl ketone
IMIS[21]:	A624
CAS number:	513-86-0 (monomer)
boiling point:	148 ºC (298 ºF) @ 760 mmHg (monomer)
melting point:	15 ºC (59 ºF) (monomer); 91 ºC (196 ºF) (dimer)
density:	1.005 (g/mL@ 25 ºC) (monomer)
molecular weight:	88.11 (monomer)
flash point:	46.7 ºC (116 ºF) (closed cup) (monomer)
appearance:	Pale yellow to colorless as liquid or solid
molecular formula:	$C_4H_8O_2$ (monomer); $C_8H_{16}O_4$ (dimer)

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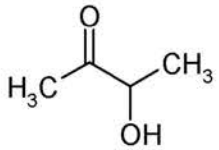
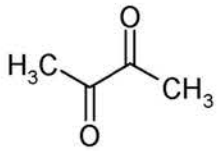
[14] Burdock, G. A. *Fenaroli's Handbook of Flavor Ingredients*, 5th ed.; CRC Press: Boca Raton, FL, 2005; pp 11-12.
[15] Burdock, G. A. *Fenaroli's Handbook of Flavor Ingredients*, 5th ed.; CRC Press: Boca Raton, FL, 2005; pp 411-412.
[16] Kanwal, R.; Boylstein, R. J.; Piacitelli, C. NIOSH Health Hazard Evaluation Report #2001-0474-2943, 2004. Centers for Disease Control and Prevention, National Institute of Occupational Safety and Health Web site. http://www.cdc.gov/niosh/hhe/reports/pdfs/2001-0474-2943.pdf (accessed July 2008) pp 8-9.
[17] Kanwal, R. Bronchiolitis obliterans in workers exposed to flavoring chemicals. *Curr Opin Pulm Med*. **2008**, 14 (2), 141-6.
[18] Kreiss, K. Flavoring-related bronchiolitis obliterans. *Curr Opin Allergy Clin Immunol* **2007**, 7 (2), 162-167.
[19] *The Merck Index*; 12th ed.; Budavari, S., Ed.; Merck & Co. Inc.: Whitehouse Station, NJ, 1996; p 12.
[20] Material Safety Data Sheet: Acetoin, 2008. The Good Scents Company Web site. http://www.thegoodscentscompany.com/msds/md102388.html (accessed July 2008).
[21] Acetyl Methyl Carbinol (Chemical Sampling Information), 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/chemicalsampling/data/CH_217010.html (accessed June 2008).

solubility:	miscible with water and alcohol; sparingly soluble in ether and petroleum ether
structural formula:	



Diacetyl[22,23]

synonyms:	biacetyl; 2,3-butanedione; 2,3-butadione; 2,3-diketobutane; dimethyl diketone; dimethylglyoxal; glyoxal, dimethyl-; 2,3-diketobutane
IMIS[24]:	D740
CAS number:	431-03-8
boiling point:	88 °C (190 °F)
melting point:	3-4 °C (37.4-39.2 °F)
density:	0.99 (g/mL@ 15/15)
molecular weight:	86.09
vapor pressure:	7 kPa @ 20 °C
flash point:	26.7 °C (80 °F) (closed cup)
appearance:	yellow to yellow-green liquid
vapor density:	3 (air = 1)
molecular formula:	$C_4H_6O_2$
odor:	quinone odor in higher concentrations, butter in lower concentrations
solubility:	4 parts water; miscible with alcohol, ether
autoignition temperature:	285 °C (545 °F)
structural formula:	



[22] *The Merck Index*; 12th ed.; Budavari, S., Ed.; Merck & Co. Inc.: Whitehouse Station, NJ, 1996; p 503.
[23] Material Safety Data Sheet: Diacetyl, 2007. Chemwatch; Victoria, Australia (accessed March 2008).
[24] Diacetyl (Chemical Sampling Information), 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/chemicalsampling/data/CH_231710.html (accessed 2008).

This method was evaluated according to the OSHA SLTC "Evaluation Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis"[25]. The Guidelines define analytical parameters, specify required laboratory tests, statistical calculations and acceptance criteria. The analyte air concentrations throughout this method are based on the recommended sampling and analytical parameters. Air concentrations in ppm are referenced to 25 °C and 101.3 kPa (760 mmHg).

1.2 Limit defining parameters

1.2.1 Detection limit of the analytical procedure

The detection limit of the analytical procedure is 0.017 ng for acetoin and 0.033 ng for diacetyl. These are the amount of analytes that will give a detector response that is significantly different from the response of a calibration blank. (Section 4.1)

1.2.2 Detection limit of the overall procedure

The detection limit of the overall procedure for acetoin is 0.10 µg per sample (0.0031 ppm or 0.011 mg/m^3) and 0.11 µg per sample for diacetyl (0.0034 ppm or 0.012 mg/m^3). These are the amounts spiked onto the sampler that will give a detector response that is significantly different from the response of a sampler blank. (Section 4.2)

1.2.3 Reliable quantitation limit

The reliable quantitation limit for acetoin is 0.35 µg per sample (0.011 ppm or 0.039 mg/m^3 for a TWA sample) and 0.37 µg per sample for diacetyl (0.012 ppm or 0.041 mg/m^3 for a TWA sample). These are the amounts spiked onto the sampler that will give a detector response that is considered the lower limit for precise quantitative measurements. (Section 4.2)

1.2.4 Instrument calibration

The standard error of estimate is 0.42 µg for acetoin over the range of 3.73 µg to 31.0 µg. The standard error of estimate is 0.82 µg for diacetyl over the range of 3.58 µg to 29.9 µg. These ranges correspond to approximately 0.25 to 2 times the target concentration. (Section 4.3)

1.2.5 Precision

The precision of the overall procedure at the 95% confidence level for the ambient temperature 18-day storage test (at the target concentration) is ±11.2% for acetoin and ±10.1% for diacetyl. These include an additional 5% for sampling pump variability. (Section 4.4)

1.2.6 Recovery

The recovery from samples used in a 18-day storage test remained above 88.5% for acetoin and 102.7% for diacetyl when the samples were stored at ambient temperature. (Section 4.5)

[25] Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. C. Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis, 1999. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/chromguide/chromguide.pdf (accessed November 2007).

1.2.7 Reproducibility

Six samples collected from a controlled test atmosphere were submitted for analysis by the OSHA Salt Lake Technical Center. The samples were analyzed according to a draft copy of this procedure after 20 days of storage at refrigerated temperature. No individual sample result deviated from its theoretical value by more than the precision reported in Section 1.2.5. (Section 4.6)

2. Sampling Procedure

All safety practices that apply to the work area being sampled should be followed. The sampling equipment should be attached to the worker in such a manner that it will not interfere with work performance or safety.

2.1 Apparatus

Sampler: glass tube with both ends flame sealed, 110-mm × 7-mm i.d., containing a glass fiber filter and 1 section of 20/40 mesh silica gel. From front to back, the sampling tube consists of a silane-treated glass wool plug, a glass fiber filter to collect particulate, 600 mg of silica gel and a second plug of silane-treated glass wool. The silica gel should be cleaned and dried as described in Appendix A. Sampling tubes are available for purchase through SKC, Inc. (cat. no. 226-183).

Samples are collected using a personal sampling pump calibrated, with the sampling device attached, to within ±5% of the recommended flow rate.

Use aluminum foil or a tube cover, such as SKC, Inc Tube Cover D (cat. no. 224-29D), to protect samples from light.

2.2 Reagents

None required

2.3 Technique

Immediately before sampling, break the ends off of two flame-sealed glass tubes to provide an opening approximately half the internal diameter of the tube. Wear eye protection when breaking ends. Use a tube holder to minimize the hazard of broken glass and to protect samplers from light exposure during sampling. All tubes should be from the same lot.

Connect the two silica gel sampling tubes in series, using the least amount of flexible tubing as possible between the sampling tubes, and then connect to a sampling pump with flexible tubing. The filter in the silica gel tubes should be positioned away from the sampling pump. The tube closer to the pump is used as a backup. Use a tube cover or wrap sampling tubes in aluminum foil to insure that both sampling tubes are protected from light exposure. Place the sampling tubes in a vertical position with the inlet in the breathing zone and position the sampling pump and tubing so they do not impede work performance or safety.

Draw air directly into the inlet of the sampler. The air being sampled should not pass through any hose or tubing before entering the sampler.

After sampling for the appropriate time, disconnect the tubes from the pump tubing and seal each tube with plastic end caps. Separately wrap each tube in aluminum foil and seal end-to end with a Form OSHA-21.

Submit at least one blank sample with each set of samples. Handle the blank sample in the same manner as the other samples except draw no air through it.

Record sample air volume (L), sampling time (min) and sampling rate (L/min) for each sample, along with any potential interferences on the Form OSHA-91A.

Submit the samples to the laboratory for analysis as soon as possible after sampling. If a delay is unavoidable, store the samples in a refrigerator. Ship any bulk samples separate from the air samples.

2.4 Sampler capacity (Section 4.7)

The sampling capacity of the front tube was tested by sampling a dynamically generated test atmosphere of acetoin (3.58 mg/m^3 or 0.99 ppm) and diacetyl (3.55 mg/m^3 or 1.01 ppm) with an average relative humidity of 40% at 34 °C (absolute humidity of 14.8 mg/L H_2O). The samples were collected at a sampling rate of approximately 0.05 L/min for 270 min. The 5% breakthrough sampling time was determined to be 248 min for diacetyl. No breakthrough was observed for acetoin. (Note: In order to volatilize acetoin the test atmosphere generation conditions were modified slightly for this method evaluation as described in the second paragraph of Section 4.11.)

2.5 Extraction efficiency (Section 4.8)

It is the responsibility of each analytical laboratory to determine the extraction efficiency because the adsorbent material, reagents and laboratory techniques may be different than those listed in this evaluation and influence the results.

The mean extraction efficiency for acetoin from dry silica gel over the range of RQL to 2 times the target concentration (0.33 to 31.0 µg per sample) was 92.9%. The extraction efficiency was not affected by the presence of water.

The mean extraction efficiency for diacetyl from dry silica gel over the range of RQL to 2 times the target concentration (0.38 to 29.9 µg per sample) was 99.6%. The extraction efficiency was not affected by the presence of water.

Extracted samples remain stable for at least 72 hr.

2.6 Recommended sampling time and sampling rate

Sample for up to 180 min at 0.05 L/min (9 L) to collect TWA (long-term) samples.

Sample for up to 15 min at 0.2 L/min (3 L) to collect short-term samples.

When short-term samples are collected, the air concentration equivalent to the reliable quantitation limit becomes larger. For example, the reliable quantitation limit is 0.032 ppm (0.12 mg/m^3) for acetoin and 0.035 ppm (0.12 mg/m^3) for diacetyl when 3 L are collected.

2.7 Interferences, sampling (Section 4.9)

Retention efficiency

The retention efficiency for all samples was 100.6% of theoretical for acetoin and 96.6% for diacetyl, when samplers containing approximately 8.3 µg of acetoin and 8.1 µg of diacetyl were allowed to sample 6.75 L of contaminant-free air having an average relative humidity of 40% at 35 °C (absolute humidity of 15.6 mg/L H_2O). Samples were collected at a sampling rate of 0.05 L/min.

Low humidity

The collection efficiency for all samples was 100.7% of theoretical for acetoin and 101.5% for diacetyl, when the samplers were used to sample a test atmosphere containing two times the target concentration having an average relative humidity of 8% at 33 °C (absolute humidity of 2.82 mg/L H_2O). Samples were collected at a sampling rate of 0.05 L/min for 180 min.

Low concentration

The collection efficiency for all samples was 91.8% of theoretical for acetoin and 95.6% for diacetyl, when the samplers were used to sample a test atmosphere containing approximately 0.1 times the target concentration having an average relative humidity of 42% at 33 °C (absolute humidity of 14.8 mg/L H_2O). Samples were collected at a sampling rate of 0.05 L/min for 180 min.

The collection efficiency for all samples when taking short term samples was 106% of theoretical for acetoin and 90.6% for diacetyl, when the samplers were used to sample a test atmosphere containing approximately 0.1 times the target concentration having an average relative humidity of 42% at 33 °C (absolute humidity of 14.8 mg/L H_2O). Samples were collected at a sampling rate of 0.2 L/min for 15 min.

Sampling interference

The collection efficiency for all samples was 95.5% of theoretical for acetoin and 101.8% for diacetyl, when the sampler was used to sample a test atmosphere containing approximately one times the target concentration of acetoin and diacetyl and 2.59 mg/m^3 of 2-nonanone and 1.88 mg/m^3 of 2,3-pentanedione. The test atmosphere had an average relative humidity of 38% at 34 °C (absolute humidity of 14.1 mg/L H_2O). Samples were collected at a sampling rate of 0.05 L/min for 181 min.

Sampler exposure to light, particularly sunlight, during sampling will result in degradation of both acetoin and diacetyl. The recovery for all samples was 67.0% of theoretical for acetoin and 6.43% for diacetyl, when the sampler was used to sample a test atmosphere containing approximately one times the target concentration of acetoin and diacetyl and then exposed to 3 h of direct sunlight (samples were covered during sampling). The test atmosphere had an average relative humidity of 40% at 35 °C (absolute humidity of 15.6 mg/L H_2O). Samples were collected at a sampling rate of 0.05 L/min for 180 min. See Section 4.9 for data on other light tests performed.

3. Analytical Procedure

Adhere to the rules set down in your Chemical Hygiene Plan[26]. Avoid skin contact and inhalation of all chemicals and review all appropriate MSDSs.

3.1 Apparatus

A gas chromatograph equipped with an FID. For this evaluation an Agilent Technologies 6890 Plus Gas Chromatograph equipped with a 7683 Automatic Sampler and an Agilent tapered, deactivated, split, low pressure drop liner with glass wool (catalog no. 5183-4647).

A GC column capable of separating acetoin and diacetyl from the desorption solvent, internal standard and any potential interferences. A Restek 60-m × 0.32-mm i.d. Rt_x-Volatiles (1.5-µm df) capillary column was used in this evaluation.

[26] Occupational Exposure to Hazardous Chemicals in Laboratories. *Code of Federal Regulations*, Part 1910.1450, Title 29, 2003.

An electronic integrator or other suitable means of measuring GC detector response. Waters Empower 2 Data System was used in this evaluation.

A dispenser capable of delivering 2.0 mL of desorbing solvent to prepare standards and samples. If a dispenser is not available, a 2.0-mL volumetric pipet can be used.

Amber glass vials with PTFE-lined caps. For this evaluation 2 and 4-mL vials were used.

Calibrated 10-µL and 25-µL syringes for preparing standards.

Water purifier. A Barnstead NANOpure Diamond system was used to produce 18.0 MΩ-cm DI water in this evaluation.

Water bath. A Precision Scientific (5 – 100 °C range) water bath was used in this evaluation.

A mechanical rotator. A Fisher Roto-Rack was used in this evaluation.

Class A 1-L volumetric flasks.

Class A 1-mL and 5-mL volumetric pipets.

3.2 Reagents and Standards

Acetoin ($C_4H_8O_2$), [CAS no. 513-86-0]. The acetoin (lot no. 05025DH) used in this evaluation was purchased from Sigma Aldrich (Milwaukee, WI).

Diacetyl ($C_4H_6O_2$), [CAS no. 431-03-8]. The diacetyl used in this evaluation was 97+% (lot no. 17823LD) purchased from Sigma Aldrich (Milwaukee, WI).

DI water, 18.0 MΩ-cm.

Ethyl Alcohol [CAS no. 64-17-5]. The ethyl alcohol used in this evaluation was 95% v/v (190 proof) A.C.S. spectrophotometric grade (lot no. B0513920) purchased from Acros Organics (Morris Plains, NJ).

3-Pentanone [Cas no. 96-22-0]. The 3-pentanone used in this evaluation was 99+% (lot no. HR 00231KF) purchased from Aldrich (Milwaukee, WI).

The extraction solvent used for this evaluation consisted of 0.007 µL/mL 3-pentanone in 95% v/v ethyl alcohol. The 3-pentanone was added to the ethyl alcohol as an internal standard (ISTD).

3.3 Standard preparation

Prepare a concentrated stock standard of acetoin and diacetyl in 18.0 MΩ-cm DI water and store in an amber vial or bottle. (**Note**: Acetoin is usually obtained as the solid dimmer and will convert back to the monomer when dissolved in water.) Acetoin will slowly dissolve in water, however, this process can be accelerated by placing the solution in a 60 ºC water bath for 10 min. Refrigerate the stock standard when not in use and remake once a month.

Prepare working analytical standards by injecting microliter amounts of the concentrated stock standard into amber 4-mL vials containing 2 mL of the extraction solvent delivered by the same dispenser used to extract samples. For example, to prepare a target level standard (16.25 µg/sample acetoin and 15.86 µg/sample diacetyl) , inject 13 µL of a stock standard containing 1.25 µg/µL acetoin and 1.22 µg/µL diacetyl into 2-mL of extraction solvent. Transfer working standards to 2-mL amber glass autosampler vials.

Bracket sample concentrations with standard concentrations. If upon analysis, sample concentrations fall outside the range of prepared standards, prepare and analyze additional standards to confirm instrument response, or dilute high samples with extraction solvent and reanalyze the diluted samples.

3.4 Sample preparation

Remove the plastic end caps from the front sample tube and carefully transfer the silica gel to a 4-mL amber glass vial. The sampling tube and the back of the glass fiber filter should be carefully inspected to insure that all the silica gel is transferred into the 4-mL vial. Remove the plastic end caps from the backup tube and carefully transfer the silica gel to a second 4-mL amber glass vial. If the industrial hygienist requests analysis of the front glass fiber filter, which is not normally analyzed, place the front glass wool plug and filter from the front tube into a third 4-mL vial. If analysis of filter is not requested then discard the front glass wool plug and filter. Discard the glass tubes and back glass wool plugs and back glass fiber filter.

Add 2.0 mL of extraction solution to each vial and immediately seal with PTFE-lined caps.

Note: The use of an extraction solution or internal standard other than that specified in Section 3.2 should not be used unless a full extraction efficiency study is performed using both dry and wet media as described in Section 4.8.

Place the 4-mL vials on a mechanical rotator and rotate at approximately 40 rpm for 60 min.

Transfer the extraction solution in each 4-mL vial to a 2-mL amber glass autosampler vial and seal with a PTFE-lined cap.

Analyze samples for acetoin and diacetyl as described in Section 3.5.

Note: If after analysis lower detection limits are needed samples can be derivatized and analyzed according to Section 3.4 of OSHA Method 1012[27].

3.5 Analysis

3.5.1 Analytical conditions

GC conditions

column temperature:	Initial 60 °C, hold 4 min; ramp at 15 °C/min to 135 °C, hold 0 min; ramp at 60 °C/min to 250 °C, hold 4 min
zone temperatures:	240 °C (injector); 250 °C (detector)
run time:	14.75 min
column mode:	constant pressure
column pressure:	14 psi
initial column gas flow:	3.3 mL/min (hydrogen)
injection size:	1.0 µL (2:1 split)

[27] Eide, M. Acetoin and Diacetyl (OSHA Method 1012), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1012/1012.html (accessed September 2008).

column:	Restek 60-m × 0.32-mm i.d. capillary Rt_x-Volatiles (df = 1.5-µm) or equivalent
inlet liner:	Agilent 5183-4647 or equivalent
retention times:	5.2 min (diacetyl) 8.1 min (acetoin) 7.5 min (ISTD)

FID conditions

hydrogen flow:	40 mL/min
air flow:	450 mL/min
nitrogen makeup flow:	45 mL/min

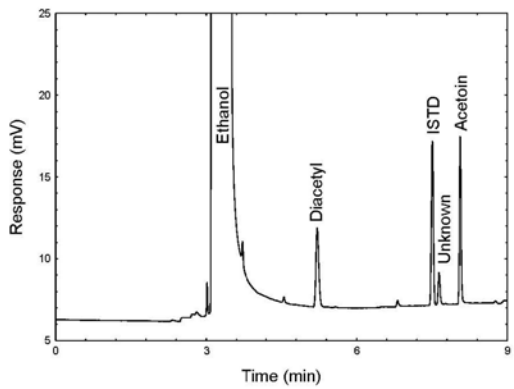


Figure 3.5.1. Chromatogram obtained at target concentrations with recommended conditions.

3.5.2 Calibration

An internal standard calibration method is used. A calibration curve can be constructed by plotting ISTD-corrected response of standard injections versus micrograms of analyte per sample. Bracket the samples with freshly prepared analytical standards over the range of concentrations.

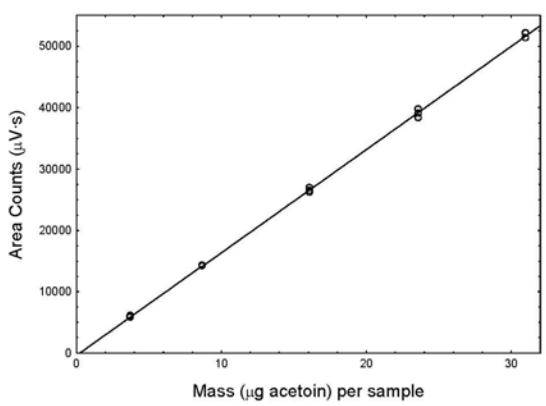


Figure 3.5.2.1. Calibration curve of acetoin. (Y = 1678X – 389)

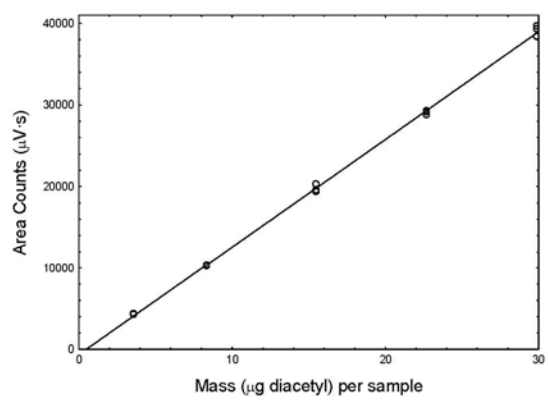


Figure 3.5.2.2. Calibration curve of diacetyl. (Y = 1320X – 625)

3.6 Interferences (analytical)

3.6.1 Any compound that produces an FID response and has a similar retention time as the analytes or internal standard is a potential interference. If any potential interferences were reported, they should be considered before samples are extracted. Generally, chromatographic conditions can be altered to separate an interference from the analyte.

3.6.2 When necessary, the identity of an analyte peak may be confirmed with additional analytical data (Section 4.12).

3.7 Calculations

The amount of analyte per sampler is obtained from the appropriate calibration curve in terms of micrograms per sample, uncorrected for extraction efficiency. The back tube is analyzed primarily to determine the extent of sampler saturation. If any analyte is found on the back tube, it is added to the amount on the front tube. This total amount is then corrected by subtracting the total amount (if any) found on the blank. The air concentration is calculated using the following formulas.

Total micrograms per sample of analyte is

$$M = [M_{front} - M_{blank}] + [M_{back} - M_{blank}]$$

where

M is total µg per sample
M_{front} is total µg found on front tube
M_{back} is total µg found on back tube
M_{blank} is total µg found on blank tube

Concentration by weight of analyte (mg/m^3) is

$$C_M = \frac{M}{E_E V}$$

where

C_M is concentration by weight (mg/m^3)
M is total µg per sample
E_E is extraction efficiency in decimal form
V is L of air sampled

Concentration by volume of analyte (ppm) is

$$C_V = \frac{V_M C_M}{M_r}$$

where

C_V is concentration by volume (ppm)
C_M is concentration by weight (mg/m^3)
V_M is molar volume at NTP (24.46 L/mole)
M_r is molecular weight (88.1 for acetoin, 86.09 for diacetyl)

4. Backup data

General background information about the determination of detection limits and precision of the overall procedure is found in the "Evaluation Guidelines for Air Sampling Methods Utilizing

Chromatography Analysis"[28]. The Guidelines define analytical parameters, specify required laboratory tests, statistical calculations and acceptance criteria.

4.1 Detection limit of the analytical procedure (DLAP)

The DLAP is measured as mass of analyte introduced onto the chromatographic column. Ten analytical standards were prepared with equally descending increments with the highest standard containing 1.10 µg/sample acetoin and 1.05 µg/sample diacetyl. This is the concentration that would produce a peak approximately 10 times the response of a calibration blank. These standards, and the calibration blank were analyzed with the recommended analytical parameters (1-µL injection with a 2:1 spit), and the data obtained were used to determine the required parameters (standard error of estimate and slope) for the calculation of the DLAP. For acetoin values of 5171 and 30 were obtained for the slope and standard error of estimate respectively. The DLAP for acetoin was calculated to be 0.017 ng acetoin.

Table 4.1.1
Detection Limit of the Analytical Procedure for Acetoin

concentration (µg/sample)	mass on column (ng)	area counts (µV·S)
0.000	0.000	0
0.110	0.028	157
0.220	0.055	224
0.330	0.083	386
0.440	0.110	515
0.550	0.138	738
0.660	0.165	818
0.770	0.193	998
0.880	0.220	1117
0.990	0.248	1248
1.100	0.275	1414

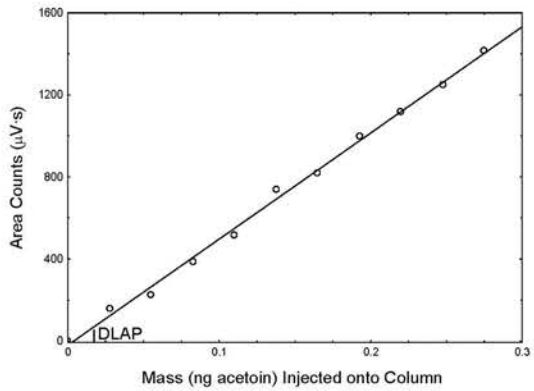


Figure 4.1.1. Plot of data to determine the DLAP for acetoin. (Y = 5171X – 19.9)

For diacetyl values of 4325 and 47 were obtained for the slope and standard error of estimate respectively. The DLAP for diacetyl was calculated to be 0.033 ng diacetyl.

Table 4.1.2
Detection Limit of the Analytical Procedure for Diacetyl

concentration (µg/sample)	mass on column (ng)	area counts (µV·S)
0.000	0.000	0
0.191	0.048	155
0.287	0.072	201
0.382	0.096	350
0.478	0.120	417
0.573	0.143	590
0.669	0.167	615
0.764	0.191	706
0.860	0.215	877
0.955	0.239	1043
1.051	0.263	1089

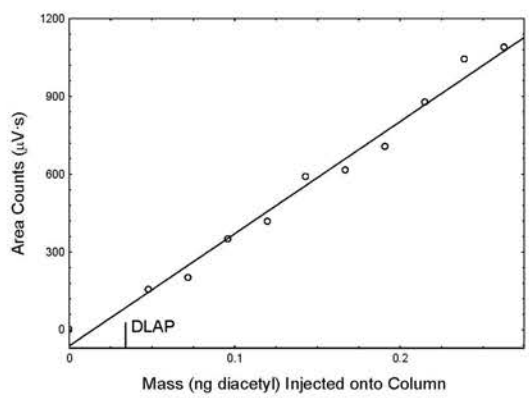


Figure 4.1.2. Plot of data to determine the DLAP for diacetyl. (Y = 4325X - 62)

[28] Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. C. Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis, 1999. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/chromguide/chromguide.pdf (accessed November 2007).

4.2 Detection limit of the overall procedure (DLOP) and reliable quantitation limit (RQL)

The DLOP is measured as mass per sample and expressed as equivalent air concentrations, based on the recommended sampling parameters. Ten samplers were spiked with equally descending increments of acetoin and diacetyl, such that the highest sampler loading was equivalent to 1.10 µg of acetoin per sample and 0.96 µg of diacetyl per sample. This is the amount spiked on a sampler that would produce a peak approximately 10 times the response of a calibration blank. These spiked samplers, and the sample blank were analyzed with the recommended analytical parameters (1-µL injection with a 2:1 spit), and the data obtained were used to determine the required parameters (slope and standard error of estimate) for the calculation of the DLOP. For acetoin values of 1029 and 36 were obtained for the slope and standard error of estimate respectively. The DLOP was calculated to be 0.10 µg acetoin per sample (0.0031 ppm or 0.011 mg/m^3 for a TWA sample).

Table 4.2.1
Detection Limit of the Overall Procedure for Acetoin

mass per sample (µg/sample)	area counts (µV·s)
0.000	0
0.110	119
0.220	226
0.330	316
0.440	432
0.550	517
0.660	605
0.770	771
0.880	848
0.990	1057
1.100	1150

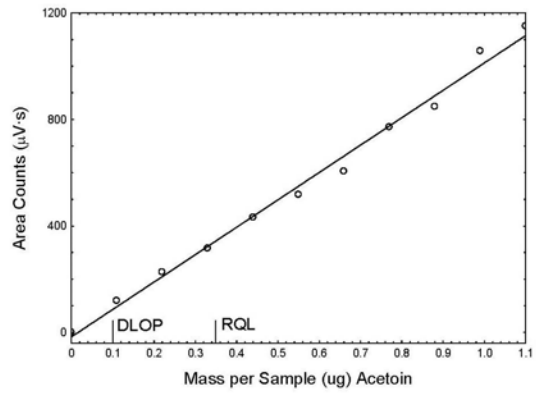


Figure 4.2.1. Plot of data to determine the DLOP/RQL for acetoin. (Y = 1029X – 16.8)

For diacetyl values of 1241 and 46 were obtained for the slope and standard error of estimate respectively. The DLOP was calculated to be 0.11 µg diacetyl per sample (0.0034 ppm or 0.012 mg/m^3 for a TWA sample).

Table 4.2.2
Detection Limit of the Overall Procedure for Diacetyl

mass per sample (µg/sample)	area counts (µV·s)
0.000	0
0.096	118
0.191	214
0.287	357
0.382	515
0.478	623
0.573	744
0.669	916
0.764	864
0.860	1043
0.955	1208

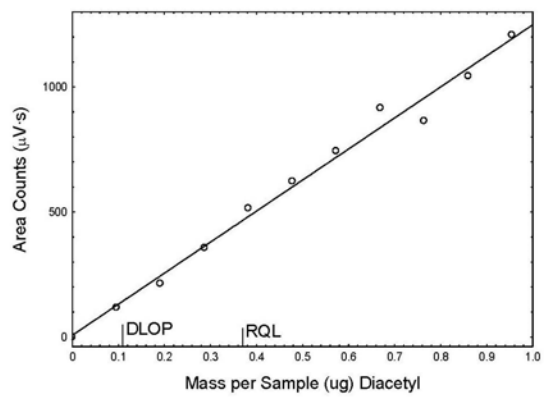


Figure 4.2.2. Plot of data to determine the DLOP/RQL for diacetyl. (Y = 1241X – 7.3)

The RQL is considered the lower limit for precise quantitative measurements. It is determined from the regression line parameters obtained for the calculation of the DLOP, providing 75% to

125% of the analyte is recovered. The RQL for acetoin is 0.35 µg per sample (0.011 ppm or 0.039 mg/m^3 for a TWA sample). Recovery at this concentration is 102%. The RQL for diacetyl is 0.37 µg per sample (0.012 ppm or 0.041 mg/m^3 for a TWA sample). Recovery at this concentration is 93.5%.

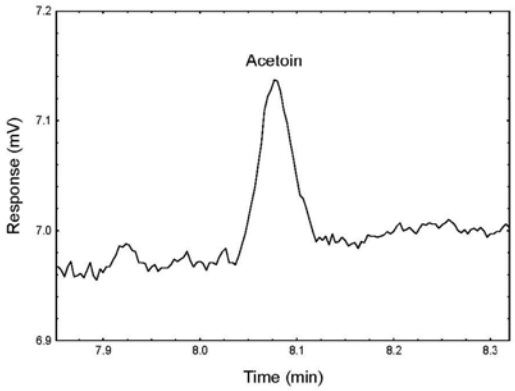


Figure 4.2.3. Chromatogram of acetoin at the RQL.

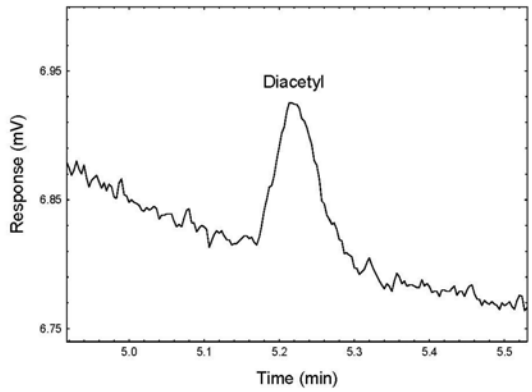


Figure 4.2.4. Chromatogram of diacetyl at the RQL.

4.3 Instrument calibration

The standard error of estimate was determined from the linear regression of data points from standards over a range that covers approximately 0.25 to 2 times the target concentration. Calibration curves for acetoin and diacetyl were constructed and are shown in Section 3.5.2 from the three injections of five standards. The standard error of estimate is 0.42 µg/sample for acetoin and 0.82 µg/sample for diacetyl.

Table 4.3.1
Acetoin Instrument Calibration

standard concn (µg/sample)	area counts (µV·s)		
3.73	5782	6047	6004
8.69	14230	14168	14323
16.1	26940	26458	26198
23.6	38318	39021	39714
31.0	52053	51292	52127

Table 4.3.2
Diacetyl Instrument Calibration

standard concn (µg/sample)	area counts (µV·s)		
3.58	4242	4347	4352
8.36	10205	10350	10373
15.5	20275	19361	19540
22.7	28772	29121	29255
29.9	39287	38363	39653

4.4 Precision (overall procedure)

The precision at the 95% confidence level is obtained by multiplying the standard error of estimate by 1.96 (the z-statistic from the standard normal distribution at the 95% confidence level). In Section 4.5, 95% confidence intervals are drawn about their respective regression lines in the storage graph figures. For acetoin the precision of the overall procedure of ±11.2% was obtained from the standard error of estimate of 5.73% in Figure 4.5.1. For diacetyl the precision of the overall procedure of ±10.1% was obtained from the standard error of estimate of 5.15% in Figure 4.5.3. The precision includes an additional 5% for sampling error.

4.5 Storage test

Storage samples for acetoin and diacetyl were prepared by collecting samples from a controlled test atmosphere using the recommended sampling conditions. The concentration of acetoin and diacetyl were at the target concentration with an average relative humidity of 41% at 34 °C (absolute humidity of 15.2 mg/L H_2O). Thirty-three storage samples were prepared. Three samples were analyzed on the day of generation. Fifteen of the samples were stored at reduced temperature (3 °C) and the other fifteen were stored in a closed drawer at ambient temperature (about 21 °C). At 3-4 day intervals, three samples were selected from each of the two storage sets and analyzed. Sample results were not corrected for extraction efficiency.

Table 4.5.1
Storage Test for Acetoin

time (days)	ambient storage recovery (%)			refrigerated storage recovery (%)		
0	86.9	87.7	89.8	86.9	87.7	89.8
4	83.1	92.0	88.3	88.0	86.1	87.4
7	91.8	85.1	90.4	95.3	90.0	94.0
11	89.1	92.3	90.9	90.6	91.4	92.1
14	90.9	88.5	91.5	90.7	88.5	91.9
18	86.5	85.5	86.1	91.7	87.6	89.9

Table 4.5.2
Storage Test for Diacetyl

time (days)	ambient storage recovery (%)			refrigerated storage recovery (%)		
0	100.5	99.9	100.7	100.5	99.9	100.7
4	98.6	100.9	100.3	97.4	96.2	98.7
7	102.6	100.9	101.2	101.5	98.8	100.9
11	102.7	104.8	101.6	101.9	101.9	102.4
14	101.9	101.0	102.7	100.2	98.8	103.2
18	101.1	103.8	101.9	100.7	98.4	102.8

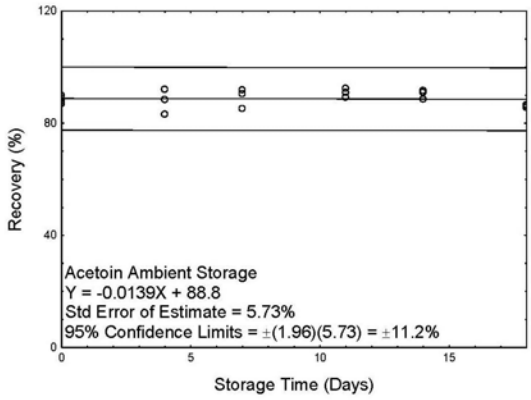


Figure 4.5.1. Ambient storage test for acetoin.

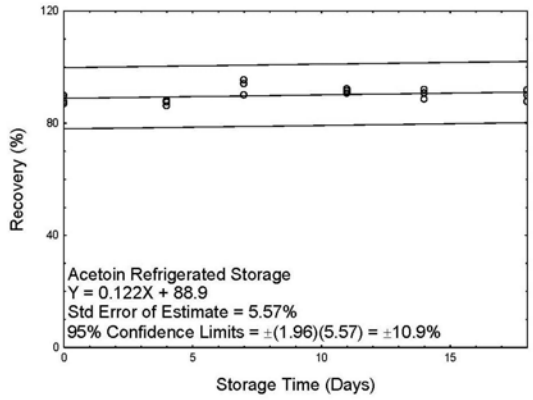


Figure 4.5.2. Refrigerated storage test for acetoin.

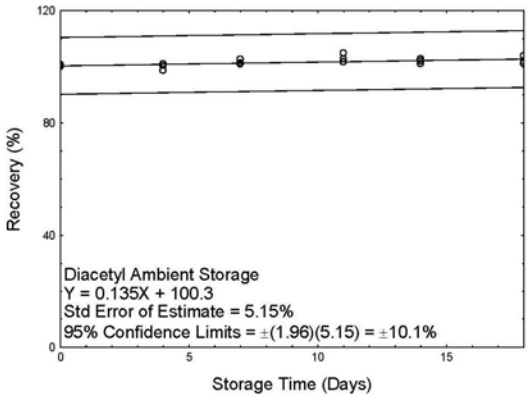


Figure 4.5.3. Ambient storage test for diacetyl.

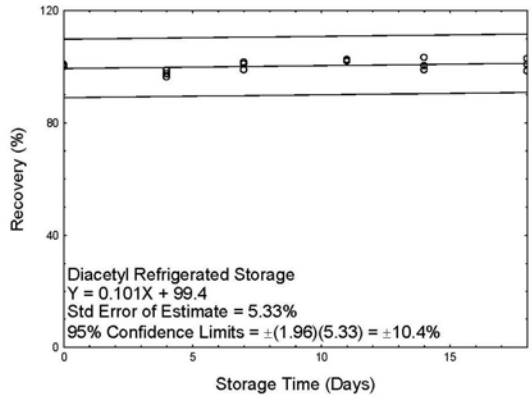


Figure 4.5.4. Refrigerated storage test for diacetyl.

4.6 Reproducibility

Six samples were prepared by collecting them from a controlled test atmosphere similar to that which was used in the collection of the storage samples. The samples were submitted to the OSHA Salt Lake Technical Center for analysis along with a draft copy of this method. The samples were analyzed after being stored for 20 days at refrigerated temperature (about 3 °C). Sample results were corrected for extraction efficiency. No sample result for acetoin and diacetyl had a deviation greater than the precision of the overall procedure determined in Section 4.4.

Table 4.6.1
Reproducibility Data for Acetoin

theoretical (µg/sample)	recovered (µg/sample)	recovery (%)	deviation (%)
16.3	17.3	106.1	6.1
16.4	15.8	96.3	-3.7
16.1	16.8	104.3	4.3
15.8	15.2	96.2	-3.8
16.1	15.7	97.5	-2.5
16.6	16.0	96.4	-3.6

Table 4.6.2
Reproducibility Data for Diacetyl

theoretical (µg/sample)	recovered (µg/sample)	recovery (%)	deviation (%)
15.9	16.6	104.4	4.4
15.9	16.3	102.5	2.5
15.7	16.5	105.1	5.1
15.4	15.8	102.6	2.6
15.7	16.0	101.9	1.9
16.2	16.6	102.5	2.5

4.7 Sampler capacity

The sampling capacity of the front tube was tested by sampling from a dynamically generated test atmosphere at 2 times the target concentration of acetoin (3.58 mg/m^3 or 0.99 ppm) and diacetyl (3.55 mg/m^3 or 1.01 ppm) with an average relative humidity of 40% at 34 °C (absolute humidity of 14.8 mg/L H_2O). The samples were collected at a sampling rate of 0.05 L/min. Backup tubes were placed in-line behind the front tube and were changed regularly after the initial collection of 225 min. Breakthrough for diacetyl was observed after sampling 12.4 L. No breakthrough was observed for acetoin even after sampling for 265 min. The recommended sampling time is 3 h.

Table 4.7
Breakthrough of Diacetyl

test no.	air vol (L)	sampling time (min)	downstream concn (mg/m^3)	breakthrough (%)
1	11.1	225	0.00	0.00
	11.8	240	0.00	0.00
	12.1	245	0.00	0.00
	12.3	250	0.00	0.00
	12.6	255	0.06	1.55
	12.8	260	0.24	6.68
	13.0	265	0.61	17.2
2	12.0	225	0.00	0.00
	12.7	240	0.22	6.32
	13.0	245	0.49	13.8
	13.3	250	0.90	25.3
	13.5	255	1.36	38.3
	13.8	260	1.86	52.3
	14.1	265	2.05	57.7
3	11.6	225	0.00	0.00
	12.4	240	0.25	7.04
	12.6	245	0.66	18.7
	12.9	250	1.32	37.0
	13.1	255	1.96	55.1
	13.4	260	2.36	66.5
	13.6	265	2.96	75.8

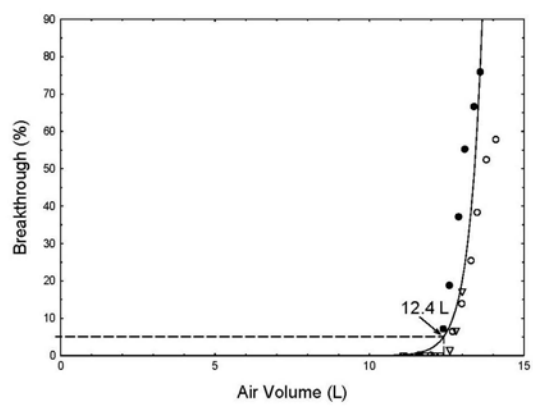


Figure 4.7. Five percent breakthrough air volume for diacetyl.

4.8 Extraction efficiency and stability of extracted samples

The extraction efficiency is dependent on the extraction solvent as well as the internal standard. Other extraction solvents or internal standards may be used provided that the new extraction solution or internal standard is tested. The new extraction solvent or internal standard should be tested as described below.

Extraction efficiency

The extraction efficiency of acetion and diacetyl was determined by liquid spiking four samplers, at each concentration level, with the analytes from the RQL to 2 times the target concentrations. These samples were stored overnight at ambient temperature and then analyzed. The mean extraction efficiency over the working range of the RQL to 2 times the target concentration is 92.9% for acetoin. The extraction efficiency for the wet samplers was not included in the overall mean because it would bias the results.

Table 4.8.1
Extraction Efficiency (%) of Acetoin

level		sample number				
x target concn	µg acetoin per sample	1	2	3	4	mean
RQL	0.33	94.0	96.5	97.4	96.7	96.2
0.25	3.73	90.5	87.8	90.1	90.5	89.7
0.5	8.69	90.2	92.4	94.6	95.6	93.2
1.0	16.2	93.2	93.7	91.9	92.6	92.8
1.5	23.6	92.3	93.6	93.5	92.0	92.8
2.0	31.0	92.7	93.8	92.7	92.5	92.9
1.0 (wet)	16.2	96.8	94.5	95.3	95.0	95.4

The mean extraction efficiency over the working range of the RQL to 2 times the target concentration is 99.6% for diacetyl. The extraction efficiency for the wet samplers was not included in the overall mean because it would bias the results.

Table 4.8.2
Extraction Efficiency (%) of Diacetyl

level		sample number				
x target concn	µg diacetyl per sample	1	2	3	4	mean
RQL	0.38	94.1	97.5	101.2	89.9	95.7
0.25	3.58	96.8	97.9	99.3	98.4	98.1
0.5	8.36	101.8	100.4	101.9	101.6	101.4
1.0	15.5	98.0	101.4	100.2	101.7	100.3
1.5	22.7	100.9	102.2	101.4	100.5	101.2
2.0	29.9	100.9	101.2	100.7	100.4	100.8
1.0 (wet)	15.5	97.8	97.3	97.2	99.7	98.0

Stability of extracted samples

The stability of extracted samples was investigated by reanalyzing the target concentration samples 24 h and 72 h after initial analysis. After each analysis was performed, two vials were recapped with new septa while the remaining two retained their punctured septa. The samples were reanalyzed with fresh standards. Samples were stored at ambient temperature and each septum was punctured 4 times for each analysis.

The average percent change for acetoin samples after 24 h was +0.5% for samples that were resealed with new septa and +0.5% for those that retained their punctured septa. The test was performed at room temperature (about 21 °C).

Table 4.8.3
24 Hour Stability of Extracted Samples for Acetoin

punctured septa replaced			punctured septa retained		
initial (%)	after one day (%)	difference (%)	initial (%)	after one day (%)	difference (%)
93.2	93.1	-0.1	91.9	92.9	+1.0
93.7	94.7	+1.0	92.6	92.5	-0.1
	(mean)			(mean)	
93.4	93.9	+0.5	92.2	92.7	+0.5

The average percent change for acetoin samples after 72 h was -1.8% for samples that were resealed with new septa and -0.9% for those that retained their punctured septa.

Table 4.8.4
72 Hour Stability of Extracted Samples for Acetoin

punctured septa replaced			punctured septa retained		
initial (%)	after one day (%)	difference (%)	initial (%)	after one day (%)	difference (%)
93.2	91.5	-1.7	91.9	91.3	-0.6
93.7	91.8	-1.9	92.6	91.3	-1.3
	(mean)			(mean)	
93.4	91.6	-1.8	92.2	91.3	-0.9

The average percent change for diacetyl after 24 h was +0.4% for samples that were resealed with new septa and -1.4% for those that retained their punctured septa. The test was performed at room temperature (about 21 °C).

Table 4.8.5
24 Hour Stability of Extracted Samples for Diacetyl

punctured septa replaced			punctured septa retained		
initial (%)	after one day (%)	difference (%)	initial (%)	after one day (%)	difference (%)
98.0	99.0	+1.0	100.2	99.5	-0.7
101.4	101.2	-0.2	101.7	99.7	-2.0
	(mean)			(mean)	
99.7	100.1	+0.4	101.0	99.6	-1.4

The average percent change for diacetyl samples after 72 h was +1.0% for samples that were resealed with new septa and -0.8% for those that retained their punctured septa.

Table 4.8.6
72 Hour Stability of Extracted Samples for Diacetyl

punctured septa replaced			punctured septa retained		
initial (%)	after one day (%)	difference (%)	initial (%)	after one day (%)	difference (%)
98.0	99.8	+1.8	100.2	100.7	+0.5
101.4	101.5	+0.1	101.7	99.7	-2.0
	(mean)			(mean)	
99.7	100.6	+1.0	101.0	100.2	-0.8

4.9 Interferences (sampling)

Retention

The ability of the sampler to retain acetoin and diacetyl was tested by sampling from a dynamically generated test atmosphere of acetoin (3.67 mg/m^3 or 1.02 ppm) and diacetyl (3.58 mg/m^3 or 1.02 ppm) with an average relative humidity of 40% at 35 °C (absolute humidity of 15.6 mg/L H_2O). Six samplers had contaminated air drawn through them at 0.05 L/min for 45 min. Sampling was discontinued and three samples set aside (first set). The generation system was flushed with contaminant-free air. Sampling resumed with the other three samples having contaminant-free air drawn through them at 0.05 L/min for 135 min and then all six samplers were analyzed. The mean of the samples in the second set had retained 100.6% for acetoin and 96.6% for diacetyl of the mean collected by the first three samples.

Table 4.9.1
Retention Efficiency (%) of Acetoin

set no.	1	2	3	mean
first	93.6	92.5	99.8	95.3
second	94.0	95.6	98.1	95.9
second/first				100.6

Table 4.9.2
Retention Efficiency (%) of Diacetyl

set no.	1	2	3	mean
first	108.0	103.0	108.5	106.5
second	102.4	102.3	103.9	102.9
second/first				96.6

Low humidity

The ability of the sampler to collect acetoin and diacetyl from a relatively dry atmosphere was tested by sampling from a dynamically generated test atmosphere of acetoin (4.06 mg/m^3 or 1.13 ppm) and diacetyl (4.03 mg/m^3 or 1.14 ppm) with an average relative humidity of 8% at 33 °C (absolute humidity of 2.82 mg/L H_2O). Three samplers had contaminated air drawn through them at 0.05 L/min for 180 min. All of the samples were immediately analyzed. The samplers collected 103.0%, 96.9% and 102.2% of theoretical for acetoin and 96.7%, 106.6% and 101.2% of theoretical for diacetyl.

Low concentration

The ability of the sampler to collect acetoin and diacetyl at low concentrations was tested by sampling from a dynamically generated test atmosphere of 0.1 times the target concentration of acetion (0.185 mg/m^3 or 0.0515 ppm) and diacetyl (0.175 mg/m^3 or 0.0497 ppm) with an average relative humidity of 42% at 33 °C (absolute humidity of 14.8 mg/L H_2O). Three samplers had contaminated air drawn through them at 0.05 L/min for 180 min. All of the samples were immediately analyzed. The samplers collected 93.9%, 91.5% and 89.9% of theoretical for acetoin and 92.8%, 97.4% and 96.7% of theoretical for diacetyl.

The ability of the sampler to collect acetoin and diacetyl at low concentrations when taking short term samples was tested by sampling from a dynamically generated test atmosphere of 0.1 times the target concentration of acetion (0.185 mg/m^3 or 0.0514 ppm) and diacetyl (0.175 mg/m^3 or 0.0497 ppm) with an average relative humidity of 42% at 33 °C (absolute humidity of 14.8 mg/L H_2O). Three samplers had contaminated air drawn through them at 0.2 L/min for 15 min. All of the samples were immediately analyzed. The samplers collected 103.8%, 104.1% and 110.0% of theoretical for acetoin and 88.1%, 89.2% and 94.4% of theoretical for diacetyl.

Interferences

The ability of the sampler to collect acetoin and diacetyl was tested when other potential interferences are present by sampling an atmosphere containing 1.63 mg/m^3 (0.45 ppm) of acetoin, 1.56 mg/m^3 (0.44 ppm) of diacetyl, 2.59 mg/m^3 (0.44 ppm) of 2-nonanone and 1.88 mg/m^3 (0.44 ppm) of 2,3-pentanedione with an average relative humidity of 38% at 34 °C

(absolute humidity of 14.1 mg/L H_2O). Three samplers had contaminated air drawn through them at 0.05 L/min for 181 min. All of the samples were immediately analyzed. The samplers collected 93.2%, 96.5% and 96.8% of theoretical for acetoin and 100.6%, 100.6% and 104.1% of theoretical for diacetyl. Selection of 2-nonanone as a potential interference was based on its common use in butter flavorings used in microwave popcorn manufacturing facilities[29]. 2,3-Pentanedione was selected because it has been suggested as a possible replacement for diacetyl. (Note: The GC retention time of 2-nonanone was 14.4 min and 7.4 min for 2,3-pentanedione. For this test the GC column temperature program was slightly changed to Initial 60 °C, hold 4 min; ramp at 15 °C/min to 225 °C, hold 0 min; ramp at 60 °C/min to 250 °C, hold 4 min to allow for the elution of 2-nonanone.)

Light

The possibility of light degradation was tested for both acetoin and diacetyl on the sampling medium and in the extraction solution. For the sample medium test 12 samples were collected by sampling from a dynamically generated test atmosphere of acetoin (1.92 mg/m^3 or 0.53 ppm) and diacetyl (1.87 mg/m^3 or 0.53 ppm) with an average relative humidity of 40% at 35 °C (absolute humidity of 15.6 mg/L H_2O). The samples were collected at a sampling rate of 0.05 L/min for 3 hours. Nine of the samples were covered with aluminum foil during sampling and three were not covered. The three samples not covered and three of the covered samples were immediately analyzed after sampling. Three of the covered samples were placed under a fluorescent lamp for 24 h and the reaming three were placed outside in direct sunlight for three hours before analyzing. The samples covered during sampling and immediately analyzed after sampling had mean recoveries of 94.4% of theoretical for acetoin and 96.6% for diacetyl. The samples not covered during sampling and immediately analyzed after sampling had mean recoveries of 94.1% of theoretical for acetoin and 96.2% for diacetyl. The samples covered during sampling and then exposed to fluorescent light for 24 h before analysis had mean recoveries of 88.7% of theoretical for acetoin and 86.8% for diacetyl. The samples covered during sampling and then exposed to sunlight for 3 h before analysis had mean recoveries of 67.0% of theoretical for acetoin and 6.43% for diacetyl. This data clearly indicates that the sampler should be protected from exposure to light.

Table 4.9.3
Sampler Light Exposure Test for Acetoin

	sample number			
type of sampler light exposure	1	2	3	mean
no light exposure	94.0	97.3	92.0	94.4
3h ambient light exposure during sampling	95.0	91.3	96.0	94.1
24h direct fluorescent light exposure after sampling, none during sampling	92.5	86.4	87.1	88.7
3h direct sunlight exposure after sampling, none during sampling	79.7	63.5	63.7	67.0

Table 4.9.4
Sampler Light Exposure Test for Diacetyl

	sample number			
type of sampler light exposure	1	2	3	mean
no light exposure	95.4	97.6	96.8	96.6
3h ambient light exposure during sampling	98.0	94.9	95.8	96.2
24h direct fluorescent light exposure after sampling, none during sampling	88.4	86.1	86.0	86.8
3h direct sunlight exposure after sampling, none during sampling	5.68	7.08	6.52	6.43

To test the possibility of light degradation on extracted samples nine analytical standards at the target concentration were prepared. Six of the standards were placed in 2-mL amber glass vials and three were placed in 2-mL clear glass vials. Three of the amber vials, along with the

[29] Kanwal, R.; Boylstein, R. J.; Piacitelli, C. NIOSH Health Hazard Evaluation Report #2001-0474-2943, 2004. Centers for Disease Control and Prevention, National Institute of Occupational Safety and Health Web site. http://www.cdc.gov/niosh/hhe/reports/pdfs/2001-0474-2943.pdf (accessed July 2008) p 46.

clear glass vials were stored on the autosampler tray during the entire test while the other three amber vials were stored in the refrigerator when not being analyzed. All nine standards were analyzed eight times over a 10 day period with none of the septa being replaced during the test. With the exception of diacetyl in clear vials, acetoin and diacetyl did not degrade. This data clearly indicates that extracted samples should be protected from exposure to light. This data also indicates that acetoin and diacetyl are stable in the extraction solution for up to 9 days as long as they are stored in amber vials.

Table 4.9.5
Extracted Sample Light Exposure Test for Acetoin

	mean of 3 peak areas		
day	clear vials (ambient)	amber vials (ambient)	amber vials (refrigerated)
0	24226	23552	23485
1	24232	23642	23535
2	23693	23232	22932
3	23455	23376	23383
4	23765	23137	23050
7	24191	23973	23280
8	23734	22969	22684
9	24245	23740	23309

Table 4.9.6
Extracted Sample Light Exposure Test for Diacetyl

	mean of 3 peak areas		
day	clear vials (ambient)	amber vials (ambient)	amber vials (refrigerated)
0	20537	19789	19640
1	19037	19667	19716
2	17814	19301	19336
3	16289	19354	19723
4	15703	19026	19304
7	14603	19687	19577
8	13328	18509	19026
9	12408	19324	19606

The internal standard, 3-pentanone, was stable for up to 9 days in both the clear and ambient vials.

4.10 Diacetyl migration within sampling tubes

In the majority of solid sorbent sampling tubes used by OSHA the sampling bed and the backup bed of sorbent are placed in the same sampling tube. For diacetyl this was not possible due to the migration of diacetyl within the sampling tube during storage. To demonstrate migration fifteen tubes were packed with 600 mg of silica gel and a backup section of 200 mg silica gel separated with a glass wool plug. These fifteen tubes were used to collect samples from a dynamically generated test atmosphere of acetoin (3.35 mg/m^3 or 0.93 ppm) and diacetyl (3.17 mg/m^3 or 0.90 ppm) with an average relative humidity of 42% at 33 °C (absolute humidity of 14.8 mg/L H_2O). The samples were collected at a sampling rate of 0.05 L/min for 3 hours. Three samples were analyzed on the day of generation and the other twelve were stored in a closed drawer at ambient temperature (about 21 °C). At 3-4 day intervals, three additional samples were analyzed. After 14 days up to 13.0% of diacetyl was found to have migrated from the front to the back section of the modified sampling tube. Acetoin did not migrate within the sampling tube.

Table 4.10
Ambient Storage Diacetyl Migration Test

time (days)	diacetyl found on backup section (%)		
0	0.00	0.00	0.00
4	3.07	0.54	0.82
7	8.30	4.59	4.94
11	5.81	11.2	7.69
14	9.63	11.9	13.0

4.11 Generation of test atmospheres

A test atmosphere generator, as diagramed in Figure 4.11, was set up in a walk-in hood. House air was dried and then humidified and regulated using a Miller Nelson Model 401 Flow-Temperature-Humidity Control System. A measured flow (typically 10 µL per min) of an acetoin and diacetyl water solution was pumped through a 0.53-mm uncoated fused silica capillary tube into the inlet manifold, using a Series D ISCO Syringe Pump with Controller, and mixed with dilution air (typically 100 liters per min) coming from the Miller Nelson Control System. The inlet manifold was heated by wrapping it in heat tape, regulated with a variable autotransformer, in order to insure vaporization of acetoin. The acetoin and diacetyl gas mixture then flowed continuously into the mixing chamber (76-cm × 15-cm) and then into the sampling chamber

(56-cm × 9.5-cm). Samples were collected through sampling ports on the sampling chamber. Temperature and humidity were measured near the exit of the sampling chamber using an Omega Digital Thermo-hygrometer model RH411.

With the exception of low humidity tests OSHA normally generates test atmospheres at an average relative humidity of 80% at 22 °C resulting in an absolute humidity of 15.5 mg/L H_2O. Due to the use of heat tape on the inlet manifold, used as mentioned above to insure the vaporization of acetoin, the test atmosphere generation temperature for this evaluation was typically around 34 °C at the sampling chamber outlet, 37 °C in the middle of the sampling chamber, 45 °C at the sampling chamber inlet and 86 °C at the mixing chamber inlet. In order to maintain a humidity of 15.5 mg/L H_2O at 34 °C the relative absolute humidity was adjusted to approximately 41%.

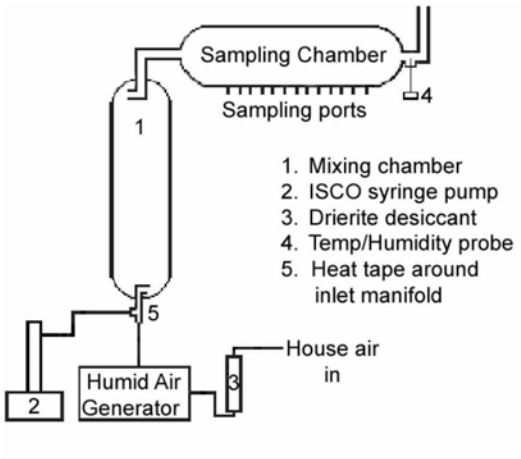


Figure 4.11. Diagram of apparatus used to generate test atmospheres.

4.12 Qualitative analysis

When necessary, the identity or purity of an analyte peak can be confirmed by GC-mass spectrometry or by another analytical procedure. The mass spectra in Figure 4.12.1 and 4.12.2 are taken from the NIST spectral library.

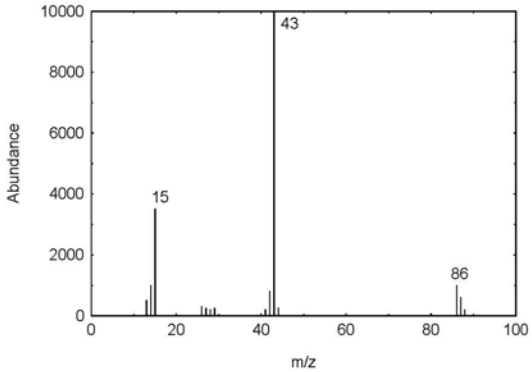


Figure 4.12.1. Mass spectrum of diacetyl.

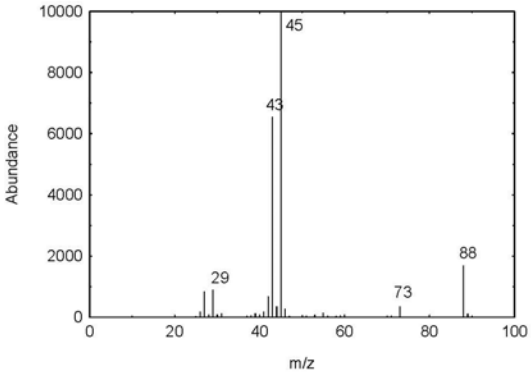


Figure 4.12.2. Mass spectrum of acetoin.

Appendix A

A.1 Silica gel preparation

For this evaluation sampling tubes were custom made by SKC, Inc. and are now available for purchase through SKC, Inc. (cat. no. 226-183).

Below are instructions on how the silica gel is prepared for the sampling tubes used in this evaluation.

A.1.1 Apparatus

Tube furnace and quartz process tube. A Lindberg model 55035 tube furnace and 1-inch diameter quartz process tube were used in this evaluation.

Nitrogen gas.

A.1.2 Silica Gel

Washed 20/40 mesh silica gel with 30 angstrom pore size (washed silica gel can be purchased from SKC, Inc.). A description of a washing procedure for silica gel can be found in the appendix of NIOSH 7903[30].

A.1.3 Preparation of silica gel

Insert a quartz wool plug in a 1-inch diameter quartz process tube, followed by 50 g of washed silica gel and a second quartz wool plug to hold the silica gel in place.

Place the process tube in a tube furnace and set the temperature to 180 °C. Continually purge the process tube with nitrogen at a rate of about 0.5 L/min. Allow the silica gel to dry in the tube furnace for 4 hours.

After 4 hours allow the process tube to cool while continuing to purge the tube with nitrogen. Once the silica gel is cool, remove one of the quartz wool plugs, and transfer silica gel into an airtight container.

[30] Cassinelli, M. E. Acids, Inorganic (NIOSH Method 7903), 1994. Centers for Disease Control and Prevention, National Institute of Occupational Safety and Health Web Site. http://www.cdc.gov/niosh/nmam/pdfs/7903.pdf (accessed July 2008).

Appendix D

2, 3-Pentanedione

2,3-Pentanedione

Method no.:	1016
Version:	1.0
Target concentration:	0.5 ppm (2.05 mg/m^3) (TWA)
Procedure:	Active samples are collected by drawing workplace air through specially dried silica gel tubes with personal sampling pumps. Samples are extracted with 95:5 ethyl alcohol:water and analyzed by gas chromatography using a flame ionization detector (GC-FID).
Recommended sampling time and sampling rate:	200 min at 50 mL/min (10.0 L) (TWA); 15 min at 0.2 L/min (3 L) (short term) 180 min at 50 mL/min (9.0 L) (TWA); 15 min at 0.2 L/min (3 L) (short term) if sampling for acetoin and diacetyl along with 2,3-pentanedione
Reliable quantitation limit:	9.3 ppb (38 µg/m^3)
Standard error of estimate at the target concentration:	10.1%
Special requirements:	Protect samplers from the light exposure during sampling, shipping, and analysis. Samples should be kept cold and shipped cold to the lab as soon as possible after sampling, preferably by overnight or express shipping. Samples should be analyzed within 17 days of sampling.
Status of method:	Fully validated method. This method has been subjected to the established validation procedures of the Methods Development Team.

July 2010

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Methods Development Team
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1. General Discussion

For assistance with accessibility problems in using figures and illustrations presented in this method, please contact Salt Lake Technical Center (SLTC) at (801) 233-4900. These procedures were designed and tested for internal use by OSHA personnel. Mention of any company name or commercial product does not constitute endorsement by OSHA.

1.1 Background

1.1.1 History

OSHA is concerned about workplace exposure to 2,3-pentanedione because it is a butter flavoring agent that is sometimes substituted for diacetyl.[1] 2,3-Pentanedione is chemically similar to diacetyl and may have similar toxicological properties.[2] This work was performed because OSHA has no sampling and analytical method for 2,3-pentanedione and none was found in a literature review.

One of the main objectives of this work was to enable OSHA CSHOs to monitor workplace exposure to diacetyl, acetoin and 2,3-pentanedione simultaneously on the same sample. Because of the similarities of the chemicals, it was decided to validate existing sampling and analytical methodology specified in OSHA Method 1013[3] for 2,3-pentanedione. That method requires sampling with two commercially available silica gel tubes connected in series. This method specifies a different GC column than specified in Method 1013 in order to optimize the analytical separation. The reliable quatitation limits for acetoin and diacetyl cited in OSHA Method 1013 were confirmed with the GC column used in this validation.

1.1.2 Toxic effects (This section is for information only and should not be taken as the basis of OSHA policy.)

2,3-Pentanedione is moderately toxic by ingestion, a skin irritant, and can cause eye and respiratory tract irritation.[4] The oral LD_{50} in rats is 3000 mg/kg. The skin irritation test in rabbits showed moderate irritation for an exposure of 500 mg/24h. Studies exposing rats to 118, 241, 318, or 354 ppm 2,3-pentanedione for 6 hours showed epithelial changes in the airways which increased with increasing air concentrations with necrosuppurative tracheitis in the rats exposed to 354 ppm.[5] This epithelial cell damage was found to progress post-exposure in rats sacrificed a day later. These epithelial changes included degeneration, apoptosis, necrosis, and neutrophilic inflammation.

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1 News Watch, Diacetyl. *The Synergist.* **March 2010**. American Industrial Hygiene Association Web site. http://www.aihasynergist-digital.org/aihasynergist/201003#pg39 (accessed August 2010).

2 Hubbs, A.F.; Mosely, A.E.; Goldsmith, W.T.; Jackson, M.C.; Kashon, M.L.; Battelli, L.A.; Schwegler-Berry, D.; Goravanahally, M.P.; Frazer, D.; Fedan, J.S.; Kreiss, K.; and Castranova, V. Airway Epithelial Toxicity of the Flavoring Agent, 2,3-Pentanedione. *Toxicologist* [CD-ROM] **2010**, *114*, 319.

3 Simmons, M., Hendricks, W. Acetoin and Diacetyl (OSHA Method 1013), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. https://www.osha.gov/dts/sltc/methods/validated/1013/1013.html (accessed December 2009).

4 *Sax's Dangerous Properties of Industrial Materials*, 10th ed.; Vol. 3, Lewis, R.J. Ed.; John Wiley & Sons; New York, 2000, p 2843.

5 Hubbs, A.F.; Mosely, A.E.; Goldsmith, W.T.; Jackson, M.C.; Kashon, M.L.; Battelli, L.A.; Schwegler-Berry, D.; Goravanahally, M.P.; Frazer, D.; Fedan, J.S.; Kreiss, K.; and Castranova, V. Airway Epithelial Toxicity of the Flavoring Agent, 2,3-Pentanedione. *Toxicologist* [CD-ROM] **2010**, *114*, 319.

1.1.3 Workplace exposure

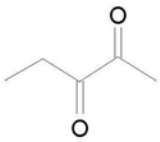
2,3-Pentanedione is a natural flavorant and odorant that is also synthesized for use in odor and flavor manufacturing.[6] It is used to give products a buttery, nutty, cheesy, fruity, toasted, chocolate, or caramel taste. It also gives products a buttery, fruity, and caramel odor. There can be as much as 58 ppm in food flavorings, and up to 0.08% in fragrances.

2,3-Pentanedione is used as a solvent for cellulose acetate, paints, inks, lacquers, as a starting material for dyes, pesticides and pharmaceuticals, and as a photoinitializer for photo-reactive dyes.[7]

1.1.4 Physical properties and other descriptive information[8,9,10]

synonyms:	acetyl propanal; acetyl propionyl; β,γ-dioxopentane; beta, gamma-dioxopentane; 2,3-pentadione		
IMIS[11]:	P110	CAS number:	600-14-6
boiling point:	110-112 °C (230-234 °F)	melting point:	-52 °C (-62 °F)
density:	0.957 g/mL @ 25 °C	molecular weight:	100.12
flash point:	19 °C (66 °F) (open cup)	molecular formula:	$C_5H_8O_2$
appearance:	yellow to yellow-green liquid	lower explosive limit:	1.8% (by volume)
autoignition temperature:	265 °C (509 °F)		
solubility:	66.7 g/L water; miscible with alcohol, fixed oils, propylene glycol		
odor:	butter-like in dilute concentration, quinone-like in high concentration		
reactive hazards:	light sensitive (Section 4.9); vapors are highly flammable and may ignite when pouring or pumping due to static electricity		

structural formula of 2,3-pentanedione



This method was validated according to the OSHA SLTC "Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis"[12]. The Guidelines define analytical parameters, specify required laboratory tests, statistical calculations, and acceptance criteria. The analyte air concentrations throughout this method are based on the recommended sampling and analytical parameters. Air concentrations in ppm are referenced to 25 °C and 760 mmHg (101.3 kPa).

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6 *Fenarolli's Handbook of Flavor Ingredients*, 5th ed.; Burdock, G.A.; CRC Press; Boca Raton, FL, 2005, p 1495.
7 2,3-Pentanedione, Chemicalland21 Website. http://chemicalland21.com/lifescience/foco/2,3-PENTANEDIONE.htm (accessed February 2010).
8 *Sax's Dangerous Properties of Industrial Materials*, 10th ed.; Vol. 3, Lewis, R.J.; John Wiley & Sons; New York, 2000, p 2843.
9 Lewis, R. J. Sr., Ed. *Hawley's Condensed Chemical Dictionary*, 14th ed.; Van Nostrand Reinhold Co.: New York, 2001, p 14.
10 3-Pentanedione(600-14-6) Chemical Book Web site. http://www.chemicalbook.com/ProductMSDSDetailCB6166470_EN.htm (accessed 1/27/2010).
11 2,3-Pentanedione (OSHA Chemical Sampling Information), 2010. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/chemicalsampling/data/CH_260240.html, (accessed 1/5/2010).
12 Eide, M.; Hendricks, W.; Simmons, M. Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis, 2010. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/chromguide/chromguide.html (accessed January 2010).

2. Sampling Procedure

All safety practices that apply to the work area being sampled should be followed. The sampling equipment should be attached to the worker in such a manner that it will not interfere with work performance or safety.

2.1 Apparatus

Samples are collected with 110-cm × 7-mm o.d. glass sampling tubes packed with a single section (600 mg) of specially cleaned and dried silica gel. The section is held in place with glass wool and with a glass fiber filter in the front and glass wool at the back. A sampling train is prepared by placing two tubes in series. For this validation, commercially prepared sampling tubes were purchased from SKC, Inc. The two tubes are identical, but SKC labels the tubes as "Part A" which is the front tube and as "Part B" which is the back tube (Catalog no. 226-183, lot no. 6148).

Use an opaque tube holder, such as SKC, Inc. Tube Cover D (cat. no. 224-29D) to cover the sampling train during sampling. If the tube holder is not opaque, wrap the sampler with aluminum foil. Light can decompose collected 2,3-pentanedione.

Samples are collected using a personal sampling pump calibrated to within ±5% of the recommended flow rate with the sampling device in-line.

2.2 Reagents

None required

2.3 Technique

Immediately before sampling, break off both ends of the flame-sealed tube to provide an opening approximately half the internal diameter of the tube. Wear eye protection when breaking ends. Use sampling tube holders to minimize the hazard to the worker from the broken ends of the tubes and to minimize the potential of glass shards entering the foodstuffs. All tubes should be from the same lot.

A sampling train is prepared by attaching a Part A tube in front of and in series with a Part B tube, with both glass fiber filters facing forward.

The Part B tube in the sampling train is used as a back-up and is positioned nearest the sampling pump. Attach the tube holder (with the adsorbent tube sampling train) to the sampling pump so that the sampling train is in an approximately vertical position with the inlet facing down in the worker's breathing zone during sampling. Position the sampling pump, tube holder and tubing so they do not impede work performance or safety.

Draw the air to be sampled directly into the inlet of the tube holder. The air being sampled is not to be passed through any hose or tubing before entering the sampling tube.

Sample for up to 200 min at 50 mL/min (10 L) to collect TWA (long-term) samples. If acetoin and/or diacetyl are anticipated to be present, sample for up to 180 min at 50 mL/min (9 L) to collect TWA (long-term) samples.

Sample for 15 min at 0.2 L/min (3 L) to collect short-term samples.

After sampling for the appropriate time, remove the sampling train, separate the tubes, and cap each tube with plastic end caps. Separately wrap each tube in aluminum foil and seal each tube end-to end with a Form OSHA-21 as soon as possible.

Submit at least one blank sample with each set of samples. Handle the blank sample in the same manner as the other samples except draw no air through it.

Record sample air volumes (L), sampling time (min), and sampling rate (mL/min) for each sample, along with any potential interferences on the Form OSHA-91A.

Submit the samples to the laboratory for analysis as soon as possible after sampling, preferably by overnight or express shipping. If delay is unavoidable, store the samples in a refrigerator. Ship samples cold to laboratory, such as shipping with frozen plastic ice packs in a cooler.

Ship any bulk samples separate from the air samples.

3. Analytical Procedure

Adhere to the rules set down in your laboratory's Chemical Hygiene Plan[13] (for instance OSHA SLTC adheres to: "The OSHA SLTC Chemical Hygiene Plan"). Avoid skin contact and inhalation of all chemicals and review all MSDSs before beginning this analytical procedure. Follow all applicable quality assurance practices established in your internal quality system (for instance OSHA SLTC follows: "The OSHA SLTC Quality Assurance Manual").

3.1 Apparatus

Gas chromatograph equipped with an FID. An Agilent 6890 GC System equipped with a Chemstation, an automatic sample injector, and an Agilent tapered, deactivated, split, low pressure drop injection port liner with glass wool (catalog no. 5183-4647) was used in this validation.

A GC column capable of separating 2,3-pentanedione from the extraction solvent, potential interferences, and internal standard. A DB-1 60-m × 0.32-mm i.d. (5-µm df) capillary column was used in this validation.

An electronic integrator or other suitable means of measuring GC detector response. A Waters Empower 2 Data System was used in this validation.

Amber glass vials with PTFE-lined caps. Two and 4-mL vials were used in this validation.

A dispenser capable of delivering 2.0 mL of extraction solvent to prepare standards and samples. If a dispenser is not available, 2.0-mL volumetric pipettes can be used.

Class A volumetric flasks - 10-mL and other convenient sizes for preparing standards.

Calibrated syringe - 25-µL and other convenient sizes for preparing standards.

Rotator. A Fisher Roto Rack was used to extract the samples in this validation.

3.2 Reagents

DI water, 18.0 MΩ-cm. A Barnstead NanoPure Diamond system was used to purify the water in this validation.

Ethyl Alcohol, [CAS no. 64-17-5]. The ethyl alcohol:water solution used in this validation was 95% v/v (190 proof) A.C.S. spectrophotometric grade (lot no. B0513920) purchased from Acros Organics (Morris Plains, NJ). Do not use absolute alcohol or denatured alcohol in this method.

[13] Occupational Exposure to Hazardous Chemicals in Laboratories. *Code of Federal Regulations*, Part 1910.1450, Title 29, 2003.

2,3-Pentanedione [CAS no. 600-14-6]. The 2,3-pentanedione used in this validation was 97% (lot no. 29598LJ) purchased from Aldrich (Milwaukee, WI).

3-Pentanone [CAS no. 96-22-0]. The 3-pentanone used in this validation was 99+% (lot no. HR 00231KF) purchased from Aldrich (Milwaukee, WI).

The extraction solvent used for this validation consisted of 0.007 µL/mL 3-pentanone in 95% v/v ethyl alcohol/water. The 3-pentanone was added to the ethyl alcohol as an internal standard (ISTD).

3.3 Standard preparation
(Note: Store all standards in amber glass bottles and vials)

Prepare concentrated stock standards in water at 1.021 mg/mL (1.021 µg/µL) by injecting 11 µL of neat 2,3-pentanedione into water in a 10-mL volumetric flask and diluting to the mark. This stock standard will remain stable for two weeks if stored in an amber bottle in the refrigerator. When using refrigerated stock standards, be sure to allow the standards to warm to room temperature and then shake them vigorously before use. Prepare analytical standards by injecting microliter amounts of concentrated stock standards into 2-mL volumetric flasks containing about 1.75 mL of extraction solvent and then diluting with extraction solvent over a concentration range of 0.1 to 20 µg/mL (0.2 to 40 µg/2 mL). For example: a target concentration standard of 20.4 µg/sample was prepared by injecting 20 µL of the stock standard into a 2-mL flask containing about 1.75 mL of extraction solvent and then diluting to the mark with extraction solvent (10.2 µg/mL or 0.5 ppm based on a 2-mL extraction volume per sample and 10 L air volumes).

Bracket sample concentrations with standard concentrations. If upon analysis, sample concentrations fall outside the range of prepared standards, prepare and analyze additional standards to confirm instrument response, or dilute high samples with extraction solvent and reanalyze the diluted samples.

3.4 Sample preparation
(Note: prepare all samples in amber glass vials)

Remove the plastic end caps from the front sample tube and carefully transfer the silica gel to a labeled 4-mL amber glass vial. The sampling tube and the back of the glass fiber filter should be carefully inspected to ensure that all the silica gel is transferred into the 4-mL vial. Remove the plastic end caps from the backup tube and carefully transfer the silica gel to a second labeled 4-mL amber glass vial. If the industrial hygienist requests analysis of the front glass fiber filter, which is not normally analyzed, place the front glass wool plug and filter from the front tube into a third 4-mL vial. If analysis of filter is not requested then discard the front glass wool plug and filter. Discard the glass tubes and back glass wool plugs and back glass fiber filter.

Add 2.0 mL of extraction solvent to each vial and immediately seal the vials with PTFE-lined caps.

Immediately place the vials on a rotator for 60 min. Transfer the sample into autosampler vials for analysis.

3.5 Analysis

3.5.1 Gas chromatographic conditions (these conditions are different from OSHA Method 1013 to obtain better separation of the 2,3-pentanedione peak from the 3-pentanone internal standard peak).

GC conditions

oven temperature:	initial 60 °C, hold 4 min, program at 10 °C/min to 150 °C, hold 5 min, 20 °C/min to 200 °C hold 1 min
injector temperature:	240 ºC
detector temperature:	250 ºC
run time:	total time is 21.5 min, data is collected for 15 min, the excess time is to clear the column
column:	60-m × 0.32-mm i.d. DB-1 capillary column (df = 5-µm)
column mode:	constant pressure
initial column gas flow:	1.8 mL/min (hydrogen)
column pressure:	9.4 psi
injection size:	1.0 µL (2:1 split)
inlet liner:	Agilent 5183-4647 or equivalent
retention times:	13.2 min 2,3-pentanedione 13.5 min 3-pentanone

FID conditions:

hydrogen flow:	40 mL/min
air flow:	450 mL/min
nitrogen makeup flow:	40 mL/min

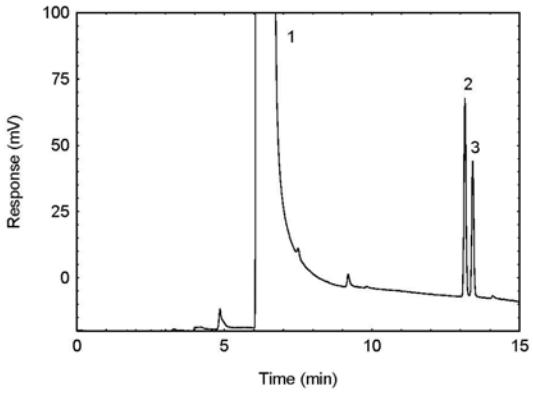

Figure 3.5.1.1. A chromatogram of 20.4 µg/sample 2,3-pentanedione. (Key: 1) ethyl alcohol; 2) 2,3-pentanedione; and 3) 3-pentanone.)

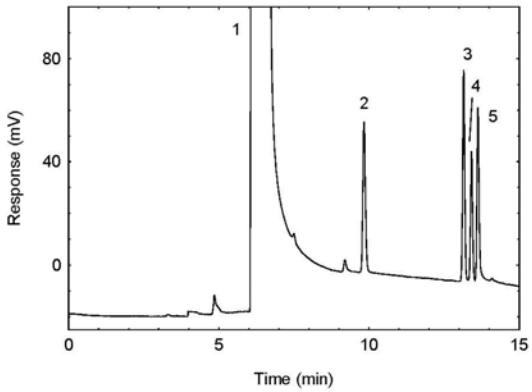

Figure 3.5.1.2. A chromatogram of 20.4 µg/sample 2,3-pentanedione, 15.8 µg/sample acetoin, and 15.6 µg/sample diacetyl. (Key: 1) ethyl alcohol; 2) diacetyl; 3) 2,3-pentanedione; 4) 3-pentanone; and 5) acetoin.)

3.5.2 An internal standard (ISTD) calibration method is used. A calibration curve can be constructed by plotting ISTD-corrected response of standard injections versus micrograms of analyte per sample. Bracket the samples with freshly prepared analytical standards over the range of concentrations.

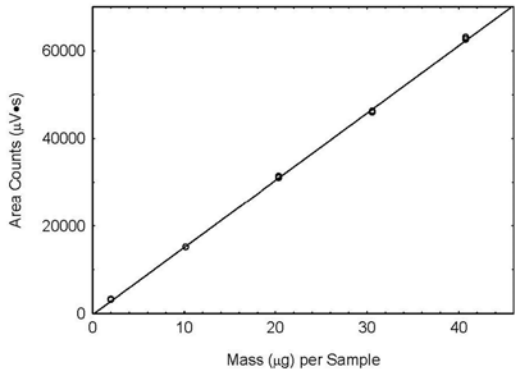

Figure 3.5.2.1. Calibration curve for 2,3-pentanedione.
(y = 1535x - 295)

3.6 Interferences (analytical)

3.6.1 Any compound that produces a GC response and has a similar retention time as the analyte or internal standard is a potential interference. If potential interferences were reported, they should be considered before samples are extracted. Generally, chromatographic conditions can be altered to separate interferences from the analyte.

3.6.2 When necessary, the identity of an analyte peak can be confirmed with additional analytical data or procedures (Section 4.10).

3.7 Calculations

The amount of analyte per sample is obtained from the appropriate calibration curve in terms of micrograms per sample, uncorrected for extraction efficiency. The second tube is analyzed primarily to determine the extent of sampler saturation. If any analyte is found on the back tube, it is added to the amount on the front tube. If more than 20% of the total amount is found on the back tube, report that the sampler may have been saturated on the Form OSHA-91B. This total amount is then corrected by subtracting the total amount (if any) found on the blank. The air concentration is calculated using the following formulas.

$$C_M = \frac{M}{VE_E}$$

where C_M is concn by weight (mg/m^3)
M is micrograms per sample
V is liters of air sampled
E_E is extraction efficiency in decimal form

$$C_V = \frac{C_M V_M}{M_r}$$

where C_V is concn by volume (ppm)
C_M is concn by weight (mg/m^3)
V_M is 24.46 (molar volume at NTP)
M_r is molecular weight of analyte
(2,3-pentanedione = 100.12)

4. Method Validation

General instruction for the laboratory validation of OSHA sampling and analytical methods that employ chromatographic analysis is presented in "Validation Guidelines for Air Sampling Methods Utilizing

Chromatography Analysis"[14]. These Guidelines detail required validation tests, show examples of statistical calculations, list validation acceptance criteria, and define analytical parameters. Air concentrations listed in ppm are referenced to 25 °C and 760 mmHg (101.3 kPa).

4.1 Detection limit of the analytical procedure (DLAP)

The DLAP is measured as mass of analyte introduced into the chromatographic column. Ten analytical standards were spiked with equally descending increments of analyte. The highest amount is the amount spiked on the sampler that would produce a peak approximately 10 times the response of a reagent blank at or near the retention time of the analyte. The standards and the reagent blank were analyzed with the recommended analytical parameters (1-µL injection with a 2:1 split). The data obtained were used to determine the required parameters (standard error of estimate and slope) for the calculation of the DLAP. The slope and standard error of estimate, respectively, were 6.62 and 62.2. The DLAP was calculated to be 28 pg.

Table 4.1
Detection Limit of the Analytical Procedure

concn (ng/mL)	mass on column (pg)	area counts (µV•s)
0	0	0
51	26	160
102	51	367
153	77	556
204	102	618
255	128	883
306	153	949
357	179	1084
408	204	1281
460	230	1547
511	256	1784

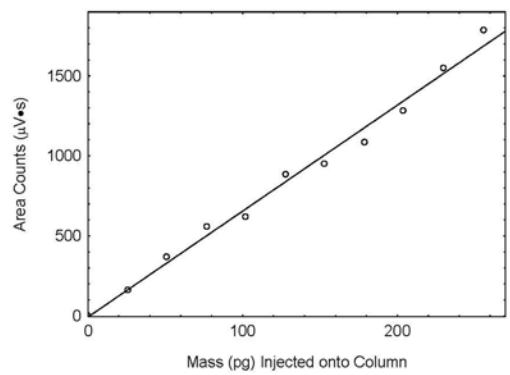

Figure 4.1. Plot of data to determine the DLAP (y = 6.62x - 7.12).

4.2 Detection limit of the overall procedure (DLOP) and reliable quantitation limit (RQL)

The DLOP is measured as mass per sample and expressed as equivalent air concentrations, based on the recommended sampling parameters. Ten samplers were spiked with equally descending increments of analyte. The highest amount is the amount spiked on the sampler that would produce a peak approximately 10 times the response of a sample blank at or near the retention time of the analyte. The spiked samplers, and the sample blank were analyzed with the recommended analytical parameters, and the data obtained used to determine the required parameters (slope and standard error of estimate) for the calculation of the DLOP. For 2,3-pentanedione values of 1597 and 61.2 were obtained for the slope and standard error of estimate respectively. The DLOP was calculated to be 0.11 µg (2.7 ppb or 11 µg/m^3).

[14] Eide, M.; Hendricks, W.; Simmons, M. *Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis.* https://www.osha.gov/dts/sltc/methods/chromguide/chromguide.pdf, OSHA Salt Lake Technical Center, U.S. Department of Labor: Salt Lake City, UT, 2010 (accessed January 2010).

Table 4.2
Detection Limit of the Overall Procedure

mass per sample (µg)	area counts (µV•s)
0.00	0
0.10	153
0.20	349
0.31	545
0.41	599
0.51	848
0.61	930
0.71	1063
0.82	1217
0.92	1485
1.02	1731

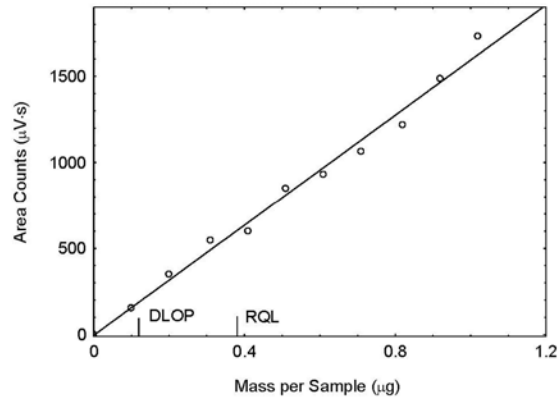

Figure 4.2.1. Plot of data to determine the DLOP/RQL (y = 1597x – 3.74).

The RQL is considered the lower limit for precise quantitative measurements. It is determined from the regression line parameters obtained for the calculation of the DLOP, providing 75% to 125% of the analyte is recovered. The RQL for 2,3-pentanedione is 0.38 µg per sample (9.3 ppb or 38 µg/m^3 for a TWA sample). Recovery at this concentration is 97.9%.

When short-term samples are collected, the air concentration equivalent to the reliable quantitation limit becomes larger. For example, the reliable quantitation limit for the recommended sampler is 31 ppb (127 µg/m^3) when 3 L is sampled.

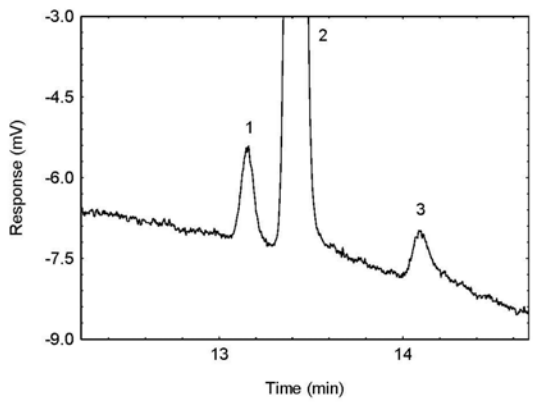

Figure 4.2.2. A chromatogram of the RQL of 2,3-pentanedione. (Key: 1) 2,3-pentanedione; 2) 3-pentanone; and 3) interferant.)

4.3 Precision of the analytical method

The precision of the analytical method was measured as the mass equivalent to the standard error of estimate determined from the linear regression of data points from standards over a range that covers 0.1 to 2 times the TWA target concentration for the sampler. A calibration curve was constructed and shown in Section 3.5.2 from the three injections each of five standards. The standard error of estimate was 0.49 µg.

Table 4.3
Instrument Calibration

x target concn (µg/sample)	0.1 x 2.04	0.5 x 10.2	1.0 x 20.4	1.5 x 30.6	2.0 x 40.8
area counts (µV·s)	3175	15104	31328	46035	62945
	3189	15132	30963	45869	63015
	3091	15094	31087	46183	62497

4.4 Storage stability test

Storage samples for 2,3-pentanedione were prepared by sampling a dynamically generated controlled test atmosphere using the recommended sampling parameters. The concentration of 2,3-pentanedione in the test atmosphere was 0.501 ppm (2.05 mg/m^3) and the relative humidity was 80% at 23 °C. Thirty-three storage samples were prepared. Three samples were analyzed on the day of generation. Fifteen of the tubes were stored at reduced temperature (4 °C) and the other fifteen were stored in a closed drawer at ambient temperature (about 23 °C). At 3 to 4-day intervals, three samples were selected from each of the two storage sets and analyzed. Sample results are not corrected for extraction efficiency. Results for the ambient storage test decreased by more than 10% which is a significant uncorrectable bias that must be avoided, therefore, samples should be stored in a refrigerator until analyzed, and analysis should be completed within two weeks of sampling. Recovery is determined from the regression line and the maximum change allowed by OSHA methods development guidelines is ±10%.

Table 4.4
Storage Test for 2,3-Pentanedione

time (days)	ambient storage recovery (%)			refrigerated storage recovery (%)		
0	95.1	96.7	97.7	95.1	96.7	97.7
4	94.4	93.2	95.4	96.6	97.5	95.9
7	91.2	93.0	94.4	97.1	94.8	96.2
10	87.8	86.9	91.4	92.5	94.3	93.0
14	85.1	84.3	86.6	90.8	92.5	93.2
17	82.4	85.0	83.9	91.4	89.5	92.7

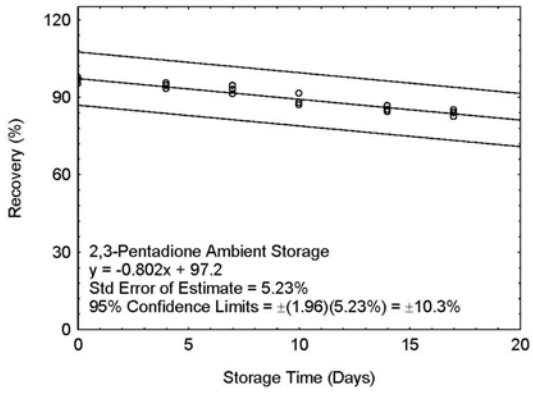

Figure 4.4.1. Ambient storage test for 2,3-pentanedione.

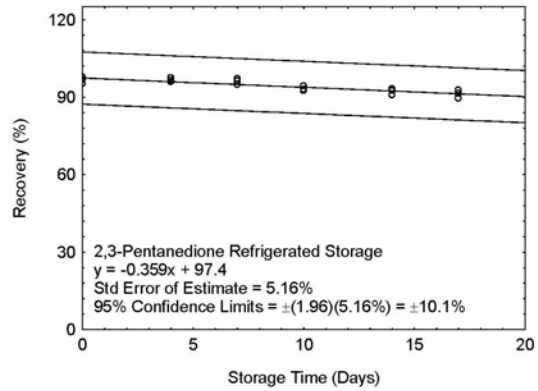

Figure 4.4.2. Refrigerated storage test for 2,3-pentanedione.

4.5 Precision (overall procedure)

The precision of the overall procedure at the 95% confidence level is obtained by multiplying the standard error of estimate by 1.96 (the z-statistic from the standard normal distribution at the 95% confidence level). Ninety-five percent confidence intervals are drawn about the regression lines in the storage stability figures shown in Section 4.4.

4.5.1 Two dried silica gel tubes in series (SKC 226-183)

The precision at the 95% confidence for the refrigerated temperature (4 °C) 17-day storage test was ± 10.1%. It contains an additional 5% for sampling pump error.

4.5.2 Recovery

The recovery of 2,3-pentanedione from samples used in a 17-day storage test remained above 91.3% when samples were stored at 4 °C.

4.6 Reproducibility

Six samples were prepared by sampling a dynamically generated controlled test atmosphere similar to that used in the collection of the storage samples. The concentrations of 2,3-pentanedione in the test atmosphere was 0.501 ppm (2.05 mg/m^3) at 78% relative humidity and 23 °C. The samples were submitted to the OSHA Salt Lake Technical Center for analysis. The samples were analyzed after being stored at 4 °C for 4 days. Sample results were corrected for extraction efficiency. No sample result had a deviation greater than the precision of the overall procedure determined in Section 4.4.

Table 4.6
Reproducibility Data

theoretical (µg/sample)	recovered (µg/sample)	recovery (%)	deviation (%)
20.5	20.0	97.6	-2.4
20.4	19.4	95.1	-4.9
21.0	19.8	94.3	-5.7
20.5	19.9	97.1	-2.9
21.0	20.3	96.7	-3.3
23.0	22.5	97.8	-2.2

4.7 Sampler capacity

The sampling capacity of the front tube of the recommended air sampler (two dried silica gel tubes in series) was tested by sampling a dynamically generated controlled test atmosphere containing 2,3-pentanedione at two times the target concentration (1.01 ppm or 4.10 mg/m^3) and 80% relative humidity at 23 °C. The samples were collected at 50 mL/min. The second tube in the sampling train was changed at 3 h then at 0.25 h intervals for the rest of the sampling. The presence of analyte on the second tube was defined as breakthrough. The percentage of the amount found on the second tube in relation to the concentration of the test atmosphere was defined as % breakthrough. The % breakthrough was plotted versus the air volume sampled to determine breakthrough air volumes. Breakthrough is considered to have occurred when the effluent from the active sampler contains a concentration of analyte that is 5% of the upstream concentration. The 5% breakthrough air volume for 2,3-pentanedione was 12.5 L. The recommended air volume is 80% of the breakthrough air volume which is 10 L (200 min sampled at 50 mL/min).

Table 4.7
Breakthrough of 2,3-Pentanedione From Front Sampling Tube of Recommended Air Sampler

test no.	air vol (L)	sampling time (min)	downstream concn mg/m^3	break-through (%)
1	9.27	180	0	0.0
	10.8	210	0	0.0
	11.6	225	0	0.0
	12.4	240	0.19	4.63
	13.2	255	2.32	56.6
2	9.06	180	0	0.0
	10.6	210	0	0.0
	11.3	225	0	0.0
	12.1	240	0.22	5.36
	12.8	255	0.67	16.3
3	8.69	180	0	0.0
	10.1	210	0	0.0
	10.9	225	0	0.0
	11.6	240	0	0.0
	12.3	255	0.18	4.59
	13.0	270	1.29	31.5

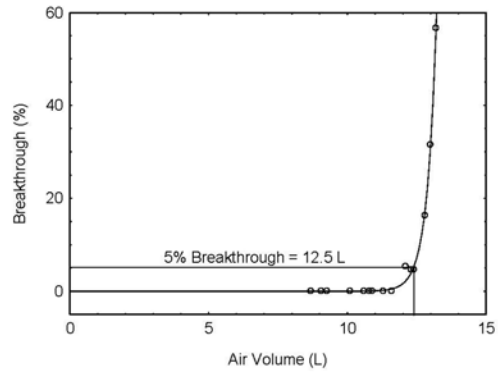

Figure 4.7. Five percent breakthrough air volume for 2,3-pentanedione.

4.8 Extraction efficiency and stability of extracted samples

The extraction efficiency is affected by the extraction solvent, the internal standard, the sampling medium, and the technique used to extract the samples. Other reagents and techniques than described in this method can be used provided they are tested as specified in the guidelines.[15]

Extraction efficiency

The extraction efficiency of 2,3-pentanedione was determined by liquid-spiking four front sampling tubes of the recommended air sampler at each concentration level. These samples were stored overnight at ambient temperature and then analyzed. The overall mean extraction efficiency over the working range of 0.1 to 2 times the target concentration was 97.6%. The presence of water had no significant effect on extraction efficiency. The extraction efficiencies for the RQL and for the wet samplers are not included in the overall mean. Wet media were prepared by sampling humid air (78% RH at 23 °C) for 200 min at 50 mL/min. The data obtained are shown in Table 4.8.1.

[15] Eide, M.; Hendricks, W.; Simmons, M. *Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis;* OSHA Web site. http://www.osha.gov/dts/sltc/methods/chromguide/chromguide.pdf (accessed 2/24/2010).

Table 4.8.1
Extraction Efficiency (%) of 2,3-Pentanedione

level		sample number				
× target concn	µg per sample	1	2	3	4	mean
0.1	2.05	98.2	97.1	96.6	98.8	97.7
0.25	5.12	97.2	98.1	95.4	96.1	96.7
0.5	10.3	98.4	95.9	97.4	97.6	97.3
1.0	20.5	96.6	96.0	97.3	98.5	97.1
1.5	30.8	98.5	98.1	98.9	96.8	98.1
2.0	40.1	97.4	99.3	99.0	98.4	98.5
RQL	0.4	98.4	96.5	97.7	99.0	97.9
1.0 (wet)	20.5	95.3	97.8	96.2	95.0	96.1

Stability of extracted samples

The stability of extracted samples was examined by reanalyzing the target concentration samples 24, 48, and 72 h after the initial analysis. After the original analysis was performed two vials were recapped with new septa which were replaced after each analysis. The remaining two vials retained their punctured septa throughout this test. All samples were allowed to stand in the autosampler tray at 22 °C. The samples were reanalyzed with freshly prepared standards. Diff is the difference between the initial analysis and the subsequent analysis. Each septum was punctured 5 times for each analysis. The data obtained are shown in Table 4.8.2.

Table 4.8.2
Stability of Extracted Samples for 2,3-Pentanedione

punctured septa replaced							punctured septa retained						
initial (%)	24 h (%)	diff (%)	48 h (%)	diff (%)	72 h (%)	diff (%)	initial (%)	24 h (%)	diff (%)	48 h (%)	diff (%)	72 h (%)	diff (%)
96.6	96.4	-0.2	96.0	-0.6	95.3	-1.3	97.3	98.7	+1.4	97.7	+0.4	95.0	-2.3
96.0	95.0	-1.0	94.8	-1.2	94.3	-1.7	98.5	97.3	-1.2	95.9	-2.6	96.4	-2.1
			(mean)							(mean)			
96.3	95.7	-0.6	95.4	-0.9	94.8	-1.5	97.9	98.0	+0.1	96.8	-1.1	95.7	-2.2

4.9 Sampling interferences

The tested sampling interferences had no significant effect on the ability of the recommended sampler to collect or retain 2,3-pentanedione when the samples were protected from exposure to light.

Retention

Retention was tested by sampling a dynamically generated controlled test atmosphere containing two times the target concentration (1 ppm or 4.1 mg/m^3) of 2,3-pentanedione at 80% relative humidity and 23 °C. The test atmosphere was sampled with the recommended sampler at 50 mL/min for 50 min. After 50 min sampling was discontinued and the samplers were separated into two sets of 3 samplers each. The generation system was flushed with contaminate-free air. Contaminant-free air is laboratory conditioned air at known relative humidity and temperature but without any added chemical except water. Sampling was resumed with a set of three samples and contaminant-free air at 80% RH and 23 °C was

sampled at 50 mL/min for 150 min and then all six samplers were analyzed. The data obtained are shown in Tables 4.9.1.

Table 4.9.1
Retention of 2,3-Pentanedione

	recovery (%)			
set	1	2	3	mean
first	98.4	100.5	98.2	99.0
second	97.7	96.8	95.1	96.5
second/first				97.5

Low humidity

The effect of low humidity was tested by sampling a dynamically generated controlled test atmosphere containing two times the target concentration (1 ppm or 4.1 mg/m^3) of 2,3-pentanedione at 20% relative humidity and 23 °C. The test atmosphere was sampled with three of the recommended samplers at 50 mL/min for 200 min. All of the samples were immediately analyzed. Sample results were 98.8%, 99.1%, and 97.4% of theoretical.

Low concentration

The effect of low concentration was tested by sampling a dynamically generated controlled test atmosphere containing 0.1 times the target concentration (0.05 ppm or 0.205 mg/m^3) of 2,3-pentanedione at 80% relative humidity and 23 °C. The test atmosphere was sampled with three of the recommended samplers at 0.05 mL/min for 200 min. All of the samples were immediately analyzed. Sample results were 98.7%, 97.0%, and 95.8% of theoretical.

Chemical interference

The ability of the recommended sampler to collect 2,3-pentanedione was tested when other potential interferences are present by sampling an atmosphere containing 0.5 ppm (2.05 mg/m^3) 2,3-pentanedione at 80% relative humidity and 23 °C and two interferences whose concentrations were 0.51 ppm (1.82 mg/m^3) acetoin, and 0.51 ppm (1.78 mg/m^3) diacetyl. The test atmosphere was sampled with three of the recommended samplers at 50 mL/min for 200 min. All of the samples were immediately analyzed. Sample results for 2,3-pentanedione were 97.1%, 96.3%, and 95.5% of theoretical.

Light

2,3-pentanedione is light-sensitive. The interference of light during sampling was tested using nine foil-wrapped samplers and three un-wrapped samplers. An atmosphere containing 0.5 ppm (2.05 mg/m^3) 2,3-pentanedione at an average humidity of 80% at 23°C was sampled for 200 minutes at 50 mL/min. The three foil-wrapped and three unwrapped samples were analyzed immediately and the average recovery for the foil wrapped was 98.2% and the un-wrapped sampler average recovery was 96.6%. Three of the foil-wrapped samplers had the foil removed after sampling and were exposed to fluorescent room lights for 24 h before analysis and had an average recovery of 90.3%. The last three foil-wrapped samplers had the foil removed and were exposed to 3 h of sunlight before analysis

Table 4.9.2
Effect of Light Exposure While Sampling

	sample number			
type of sampler light exposure	1	2	3	mean
no light exposure	97.5	98.0	99.1	98.2
200 min room light	95.1	96.8	97.9	96.6
24 h fluorescent	90.7	91.3	89.0	90.3
3 h sunlight	39.6	42.9	44.6	42.4

and had an average recovery of 42.4%. This data clearly indicates that the sampler should be protected from exposure to light.

To test the possibility of light degradation on extracted samples nine analytical standards at the target concentration were prepared. Six of the standards were placed in 2-mL amber glass vials and three were placed in 2-mL clear glass vials. Three of the amber vials, along with the clear glass vials were stored on the autosampler tray during the entire test while the other three amber vials were stored in the refrigerator when not being analyzed. All nine standards were analyzed eight times over a 10 day period with none of the septa being replaced during the test. The standards in clear vials degraded significantly, but standards in amber vials did not degrade. This data clearly indicates that extracted samples should be protected from exposure to light. The internal standard, 3-pentanone was stable for up to 9 days in both the clear and ambient vials. The data obtained is shown in Table 4.9.3.

Table 4.9.3
Extracted Sample Light Exposure Test
of 2,3-Pentanedione

	mean of peak areas from 3 vials		
day	clear vials ambient	amber vials (ambient)	amber vials (refrigerated)
0	31456	31502	31435
1	29007	31003	31354
2	27183	30961	31269
3	25072	30839	31178
4	24193	30709	31073
7	22056	30423	30834
8	20502	30389	30805
9	19584	30355	30793

4.10 Qualitative analysis

When necessary, the identity or purity of an analyte peak can be confirmed by GC-mass spectrometry or by another analytical procedure.

The mass spectrum of 2,3-pentanedione shown in Figure 4.10 was obtained by analysis on an Agilent 7890A GC System with a 5975 Mass Selective Detector.

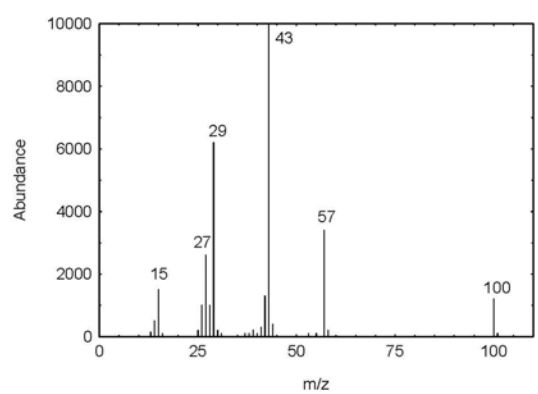

Figure 4.10. Mass spectrum of 2,3-pentanedione.

GC/MS conditions

oven temperature:	initial 35 °C, hold 5 min, program at 10 °C/min to 270 °C, hold 0 min
injector temperature:	240 °C
transfer line temperature:	250 °C
run time:	29 min
column gas flow:	1.0 mL/min (helium)
injection size:	0.5 µL (splitless)
column:	30-m × 0.25-mm i.d. DB-5 capillary column (df = 0.25 µm)

retention times: 3.8 min 2,3-pentanedione
4.2 min 3-pentanone

MS conditions

MS source temperature: 230 °C
MS quad temperature: 150 °C
Mass range: 12-250 amu

4.11 Generation of test atmospheres

The following apparatus was placed in a walk-in hood. The test atmospheres were generated by pumping low microliter volumes of a solution containing 2,3-pentanedione in water with an ISCO precision LC pump through a short length of 0.53-mm uncoated fused silica capillary tubing into a vapor generator where it was heated and evaporated into the dilution air stream (Figure 4.11). The vapor generator consisted of a 15-cm length of 5-cm diameter glass tubing with a side port for introduction of the capillary tubing. The vapor generator was heated with a variable voltage controlled heating tape to evaporate the 2,3-pentanedione. The humidity, temperature, and volume of the dilution air were regulated by use of a Miller Nelson Flow-Temperature-Humidity controller. The test atmosphere passed into a glass mixing chamber (76-cm × 30-cm) from the vapor generator, and then into a glass exposure chamber (76-cm × 20-cm). Active samplers were attached to glass ports extending from the exposure chamber. The humidity and temperature were measured at the exit of the exposure chamber with an Omega Digital Thermo-hygrometer. The theoretical concentrations were calculated from the ISCO pump flow rate, the concentration of the 2,3-pentanedione solution, and the air flow volumes. The theoretical concentrations were used throughout this validation.

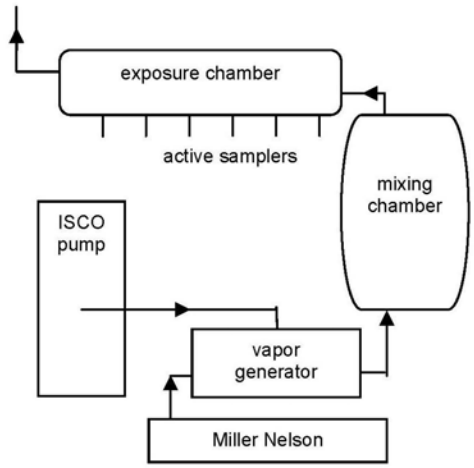

Figure 4.11. The test atmosphere generation and sampling apparatus.

Appendix E

Volatile Organic Compounds (Screening) 2549

FORMULA see Table 1 MW: see Table 1 CAS: see Table 1 RTECS: see Table 1

METHOD: 2549, Issue 1	EVALUATION: PARTIAL	Issue 1: 15 May 1996

OSHA:
NIOSH: varies with compound
ACGIH:

PROPERTIES: See Table 1

SYNONYMS: VOCs; See individual compounds in Table 1

SAMPLING

SAMPLER: THERMAL DESORPTION TUBE (multi-bed sorbent tubes containing graphitized carbons and carbon molecular sieve sorbents [See Appendix])

FLOW RATE: 0.01 to 0.05 L/min

VOL-MIN: 1 L
-MAX: 6 L

SHIPMENT: Ambient in storage containers

SAMPLE STABILITY: Compound dependent (store @ -10 °C)

BLANKS: 1 to 3 per set

ACCURACY

RANGE STUDIED: not applicable

BIAS: not applicable

OVERALL PRECISION ($\hat{S}_{rT}$): not applicable

ACCURACY: not applicable

MEASUREMENT

TECHNIQUE: THERMAL DESORPTION, GAS CHROMATOGRAPHY, MASS SPECTROMETRY

ANALYTE: See Table 1

DESORPTION: Thermal desorption

INJECTION VOLUME: Defined by desorption split flows (See Appendix)

TEMPERATURE-DESORPTION: 300 °C for 10 min.
-DETECTOR (MS): 280 °C
-COLUMN: 35 °C for 4 min; 8 °C/min to 150 °C, 15 °C/min to 300 °C

CARRIER GAS: Helium

COLUMN: 30 meter DB-1, 0.25-mm ID, 1.0-µm film, or equivalent

CALIBRATION: Identification based on mass spectra interpretation and computerized library searches.

RANGE: not applicable

ESTIMATED LOD: 100 ng per tube or less

PRECISION ($\hat{S}_r$): not applicable

APPLICABILITY: This method has been used for the characterization of environments containing mixtures of volatile organic compounds (See Table 1). The sampling has been conducted using multi-bed thermal desorption tubes. The analysis procedure has been able to identify a wide range of organic compounds, based on operator expertise and library searching.

INTERFERENCES: Compounds which coelute on the chromatographic column may present an interference in the identification of each compound. By appropriate use of background subtraction, the mass spectrometrist may be able to obtain more representative spectra of each compound and provide a tentative identity (See Table 1).

OTHER METHODS: Other methods have been published for the determination of specific compounds in air by thermal desorption/gas chromatography [1-3]. One of the primary differences in these methods is the sorbents used in the thermal desorption tubes.

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REAGENTS:

1. Air, dry
2. Helium, high purity
3. Organic compounds of interest for mass spectra verification (See Table 1).*
4. Solvents for preparing spiking solutions: carbon disulfide (low benzene chromatographic grade), methanol, etc.(99+% purity)

* See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Sampler: Thermal sampling tube, ¼" s.s. tube, multi-bed sorbents capable of trapping organic compounds in the C_3-C_{16} range. Exact sampler configuration depends on thermal desorber system used. See Figure 1 for example.
2. Personal sampling pump, 0.01 to 0.05 L/min, with flexible tubing.
3. Shipping containers for thermal desorber sampling tubes.
4. Instrumentation: thermal desorption system, focusing capability, desorption temperature appropriate to sorbents in tube (~300 °C), and interfaced directly to a GC-MS system.
5. Gas chromatograph with injector fitted with 1/4" column adapter, 1/4" Swagelok nuts and Teflon ferrules (or equivalent).
6. Syringes: 1-µL, 10-µL (liquid); 100-µL, 500-µL (gas tight)
7. Volumetric Flasks, 10-mL.
8. Gas bulb, 2 L

SPECIAL PRECAUTIONS: Some solvents are flammable and should be handled with caution in a fume hood. Precautions should be taken to avoid inhalation of the vapors from solvents as well. Skin contact should be avoided.

SAMPLING:

NOTE: Prior to field use, clean all thermal desorption tubes thoroughly by heating at or above the intended tube desorption temperature for 1-2 hours with carrier gas flowing at a rate of at least 50 mL/min. Always store tubes with long-term storage caps attached, or in containers that prevent contamination. Identify each tube uniquely with a permanent number on either the tube or tube container. Under no circumstances should tape or labels be applied directly to the thermal desorption tubes.

1. Calibrate each personal sampling pump with a representative sampler in line.
2. Remove the caps of the sampler immediately before sampling. Attach sampler to personal sampling pump with flexible tubing.
   NOTE: With a multi-bed sorbent tube, it is extremely important to sample in the correct direction, from least to maximum strength sorbent.
3. For general screening, sample at 0.01 to 0.05 L/min for a maximum sample volume of 6 L. Replace caps immediately after sampling. Keep field blanks capped at all times. Tubes can act as diffusive samplers if left uncapped in a contaminated environment.
4. Collect a "humidity test" sample to determine if the thermal adsorption tubes have a high water background.
   NOTE: At higher sample volumes, additional analyte and water (from humidity) may be collected on the sampling tube. At sufficiently high levels of analyte or water in the sample, the mass spectrometer may malfunction during analysis resulting in loss of data for a given sample.
5. Collect a "control" sample. For indoor air samples this could be either an outside sample at the same location or an indoor sample taken in a non-complaint area.
6. Ship in sample storage containers at ambient temperature. Store at -10 °C.

SAMPLE PREPARATION:

7. Allow samples to equilibrate to room temperature prior to analysis. Remove each sampler from its storage container.

8. Analyze "humidity test" sampler first to determine if humidity was high during sampling (step 10).
9. If high humidity, dry purge the tubes with purified helium at 50 to 100 mL/min for a maximum of 3 L at ambient temperature prior to analysis. .
10. Place the sampler into the thermal desorber. Desorb in reverse direction to sampling flow.

CALIBRATION AND QUALITY CONTROL:

11. Tune the mass spectrometer according to manufacturer's directions to calibrate.
12. Make at least one blank run prior to analyzing any field samples to ensure that the TD-GC-MS system produces a clean chromatographic background. Also make a blank run after analysis of heavily concentrated samples to prevent any carryover in the system. If carryover is observed, make additional blank runs until the contamination is flushed from the thermal desorber system.
13. Maintain a log of thermal desorber tube use to record the number of times used and compounds found. If unexpected analytes are found in samples, the log can be checked to verify if the tube may have been exposed to these analytes during a previous sampling use.
14. Run spiked samples along with the screening samples to confirm the compounds of interest. To prepare spiked samples, use the procedure outlined in the Appendix .

MEASUREMENT:

15. See Appendix for conditions. MS scan range should cover the ions of interest, typically from 20 to 300 atomic mass units (amu). Mass spectra can either be identified by library searching or by manual interpretation (see Table 1). In all cases, library matches should also be checked for accurate identification and verified with standard spikes if necessary.

EVALUATION OF METHOD:

The method has been used for a number of field screening evaluations to detect volatile organic compounds. Estimate of the limit of detection for the method is based on the analysis of spiked samples for a number of different types of organic compounds. For the compounds studied, reliable mass spectra were collected at a level of 100 ng per compound or less. In situations where high levels of humidity may be present on the sample, some of the polar volatile compounds may not be efficiently collected on the internal trap of the thermal desorber. In these situations, purging of the samples with 3 L of helium at 100 mL/min removed the excess water and did not appreciably affect the recovery of the analytes on the sample.

REFERENCES:

[1] Health and Safety Executive [1992]. MDHS 72 - Volatile organic compounds in air. Methods for the determination of hazardous substances. HMSO: London: ISBN 0-11-885692-8.
[2] McCaffrey CA, MacLachlan J, Brookes BI [1994]. Adsorbent tube evaluation for the preconcentration of volatile organic compounds in air for analysis by gas chromatography-mass spectrometry. Analyst 119:897-902.
[3] Bianchi AP, Varney MS [1992]. Sampling and analysis of volatile organic compounds in estuarine air by gas chromatography and mass spectrometry. J. Chromatogr. 643:11-23.
[4] EPA [1984]. Environmental Protection Agency Air Toxics Method T01. Rev. 1.0 (April, 1984): Method for the determination of volatile organic compounds in ambient air using Tenax(R) adsorption and gas chromatography/mass spectrometry (GC/MS), Section 13.

METHOD WRITTEN BY:
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TABLE 1. COMMON VOLATILE ORGANIC COMPOUNDS WITH MASS SPECTRAL DATA

Compound /Synonyms	CAS# RTECS	Empirical Formula	MW[a]	BP[b] (°C)	VP[c] @ 25 °C mm Hg	kPa	Characteristic Ions, m/z
Aromatic Hydrocarbons							
Benzene /benzol	71-43-2 CY1400000	C_6H_6	78.11	80.1	95.2	12.7	78*
Xylene /dimethyl benzene	1330-20-7 ZE2100000	C_8H_{10}	106.7				91, 106*, 105
o-xylene				144.4	6.7	0.9	
m-xylene				139.1	8.4	1.1	
p-xylene				138.4	8.8	1.2	
Toluene /toluol	108-88-3 XS5250000	C_7H_8	92.14	110.6	28.4	3.8	91, 92*
Aliphatic Hydrocarbons							
n-Pentane	109-66-0 RZ9450000	C_5H_{12}	72.15	36.1	512.5	68.3	43, 72*, 57
n-Hexane /hexyl-hydride	110-54-3 MN9275000	C_6H_{14}	86.18	68.7	151.3	20.2	57, 43, 86*, 41
n-Heptane	142-82-5 MI7700000	C_7H_{16}	100.21	98.4	45.8	6.1	43, 71, 57, 100*,41
n-Octane	111-65-9 RG8400000	C_8H_{18}	114.23	125.7	14.0	1.9	43, 85, 114*, 57
n-Decane /decyl hydride	124-18-5 HD6500000	$C_{10}H_{22}$	142.29	174	1.4	0.2	43, 57, 71, 41, 142*
Ketones							
Acetone /2-propanone	67-64-1 AL3150000	C_3H_6O	58.08	56	266	35.5	43, 58*
2-Butanone /methyl ethyl ketone	78-93-3 EL6475000	C_4H_8O	72.11	79.6	100	13	43, 72*
Methyl isobutyl ketone /MIBK, hexone	108-10-1 SA9275000	$C_6H_{12}O$	100.16	117	15	2	43, 100*, 58
Cyclohexanone /cyclohexyl ketone	108-94-1 GW1050000	$C_6H_{10}O$	98.15	155	2	0.3	55, 42, 98*, 69
Alcohols							
Methanol /methyl alcohol	67-56-1 PC1400000	CH_3OH	32.04	64.5	115	15.3	31, 29, 32*
Ethanol /ethyl alcohol	64-17-5 KQ6300000	C_2H_5OH	46.07	78.5	42	5.6	31, 45, 46*
Isopropanol /1-methyl ethanol	67-63-0 NT8050000	C_3H_7OH	60.09	82.5	33	4.4	45, 59, 43
Butanol /butyl alcohol	71-36-3 EO1400000	C_4H_9OH	74.12	117	4.2	0.56	56, 31, 41, 43

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Compound /Synonyms	CAS# RTECS	Empirical Formula	MW[a]	BP[b] (°C)	VP[c] @ 25 °C mm Hg	kPa	Characteristic Ions, m/z
Glycol Ethers							
Butyl cellosolve /2-butoxyethanol	111-76-2 KJ8575000	$C_6H_{14}O_2$	118.17	171	0.8	0.11	57, 41, 45, 75, 87
Diethylene glycol ethyl ether /Carbitol	111-90-0 KK8750000	$C_6H_{14}O_3$	134.17	202	0.08	0.01	45, 59, 72, 73, 75, 104
Phenolics							
Phenol /hydroxybenzene	108-95-2 SJ3325000	C_6H_5OH	94.11	182	47	0.35	94*, 65, 66, 39
Cresol	1319-77-3 GO5950000	C_7H_7OH	108.14				108*, 107, 77, 79
2-methylphenol	95-48-7			190.9	1.9	0.25	
3-methylphenol	108-39-4			202.2	1.0	0.15	
4-methylphenol	106-44-5			201.9	0.8	0.11	
Chlorinated Hydrocarbons							
Methylene chloride /dichloromethane	75-09-2 PA8050000	CH_2Cl_2	84.94	40	349	47	86*, 84, 49, 51
1,1,1-Trichloroethane /methyl chloroform	71-55-6 KJ2975000	CCl_3CH_3	133.42	75	100	13.5	97, 99, 117, 119
Perchloroethylene /hexachloroethane	127-18-4 KX3850000	CCl_3CCl_3	236.74	187 (subl)	0.2	<0.1	164*, 166, 168, 129, 131, 133, 94, 96
o-,p-Dichlorobenzenes		$C_6H_4Cl_2$	147.0				146*, 148, 111, 113, 75
/1,2-dichlorobenzene	95-50-1 CZ4500000			172-9	1.2	0.2	
/1,4-dichlorobenzene	106-46-7 CZ4550000			173.7	1.7	0.2	
1,1,2-Trichloro-1,2,2-trifluoroethane /Freon 113	76-13-1 KJ4000000	CCl_2FCClF_2	187.38	47.6	384	38	101, 103, 151, 153, 85, 87
Terpenes							
d-Limonene	5989-27-5 OS8100000	$C_{10}H_{16}$	136.23	176	1.2		68, 67, 93, 121, 136*
Turpentine (Pinenes)	8006-64-2	$C_{10}H_{16}$	136.23	156 to 170	4 @ 20°		93, 121, 136*, 91
α-pinene	80-56-8			156			
β-pinene	127-91-3			165			
Aldehydes							
Hexanal /caproaldehyde	66-25-1 MN7175000	$C_6H_{12}O$	100.16	131	10	1.3	44, 56, 72, 82, 41

NIOSH Manual of Analytical Methods (NMAM), Fourth Edition, 5/15/96

Compound /Synonyms	CAS# RTECS	Empirical Formula	MW[a]	BP[b] (°C)	VP[c] @ 25 °C mm Hg	kPa	Characteristic Ions, m/z
Benzaldehyde /benzoic aldehyde	100-52-7 CU4375000	$C_7H_{12}O$	106.12	179	1.0	0.1	77, 105, 106*, 51
Nonanal /pelargonic aldehyde	124-19-6 RA5700000	$C_9H_{18}O$	142.24	93	23	3	43, 44, 57, 98, 114
Acetates							
Ethyl acetate /acetic ether	141-78-6 AH5425000	$C_4H_8O_2$	88.1	77	73	9.7	43, 88*, 61, 70, 73, 45
Butyl acetate /acetic acid butyl ester	123-86-4 AF7350000	$C_6H_{12}O_2$	116.16	126	10	1.3	43, 56, 73, 61
Amyl acetate /banana oil	628-63-7 AJ1925000	$C_7H_{14}O_2$	130.18	149	4	0.5	43, 70, 55, 61
Other							
Octamethylcyclotetra-siloxane	556-67-2 GZ4397000	$C_8H_{24}O_4Si_4$	296.62	175			281, 282, 283

[a] Molecular Weight
[b] Boiling Point
[c] Vapor Pressure
* Indicates molecular ion

APPENDIX

Multi-bed sorbent tubes: Other sorbent combinations and instrumentation/conditions shown to be equivalent may be substituted for those listed below. In particular, if the compounds of interest are known, specific sorbents and conditions can be chosen that work best for that particular compound(s). The tubes that have been used in NIOSH studies with the Perkin Elmer ATD system are ¼" stainless steel tubes, and are shown in the diagram below:

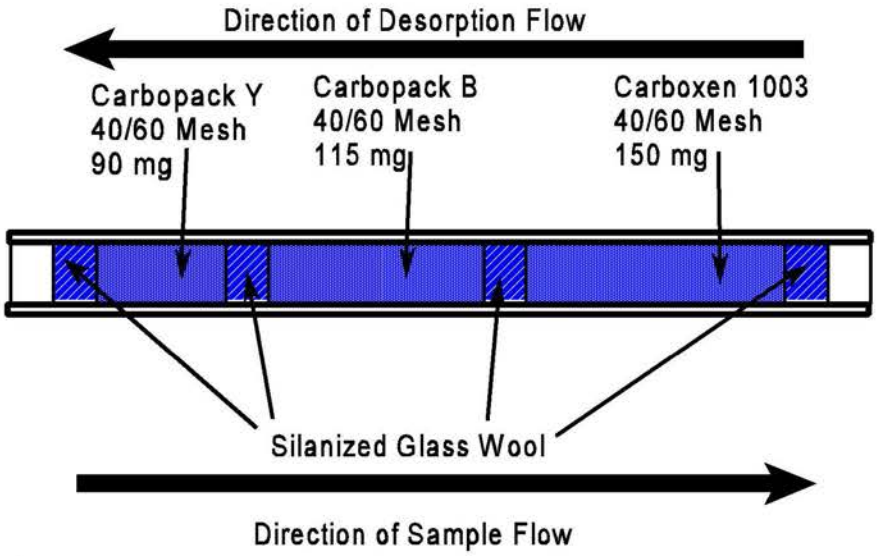

Figure 1

Carbopack™ and Carboxen™ adsorbents are available from Supelco, Inc.

Preparation of spiked samples Spiked tubes can be prepared from either liquid or gas bulb standards.

Liquid standards: Prepare stock solutions by adding known amounts of analytes to 10-mL volumetric flasks containing high purity solvent (carbon disulfide, methanol, toluene). Solvents are chosen based on solubility for the analytes of interest and ability to be separated from the analytes when chromatographed. Highly volatile compounds should be dissolved in a less volatile solvent. For most compounds, carbon disulfide is a good general purpose solvent, although this will interfere with early eluting compounds.

Gas bulb standards: Inject known amounts of organic analytes of interest into a gas bulb of known volume filled with clean air [4]. Prior to closing the bulb, place a magnetic stirrer and several glass beads are placed in the bulb to assist in agitation after introduction of the analytes. After injection of all of the analytes of interest into the bulb, warm the bulb to 50 °C and place it on a magnetic stirring plate and stir for several minutes to ensure complete vaporization of the analytes. After the bulb has been stirred and cooled to room temperature, remove aliquots from the bulb with a gas syringe and inject into a sample tube as described below.

Tube spiking: Fit a GC injector with a ¼" column adapter. Maintain the injector at 120 °C to assist in vaporization of the injected sample. Attach cleaned thermal desorption tubes to injector with ¼" Swagelok nuts and Teflon ferrules, and adjust helium flow though the injector to 50 mL/min. Attach the sampling tube so that flow direction is the same as for sampling. Take an aliquot of standard solution (gas standards 100 to 500 µL; liquid standards, 0.1 to 2 µL) and inject into the GC injector. Allow to equilibrate for 10 minutes. Remove tube and analyze by thermal desorption using the same conditions as for field samples.

Instrumentation: Actual media, instrumentation, and conditions used for general screening of unknown environments are as follows: Perkin-Elmer ATD 400 (automated thermal desorption system) interfaced directly to a Hewlett-Packard 5980 gas chromatograph/HP5970 mass selective detector and data system.

ATD conditions:

Tube desorption temperature: 300°C
Tube desorption time: 10 min.
Valve/transfer line temperatures: 150°C
Focusing trap: Carbopack B/Carboxen 1000, 60/80 mesh, held at 27°C during tube desorption
Focusing trap desorption temperature: 300°C
Desorption flow: 50-60 mL/min.
Inlet split: off
Outlet split: 20 mL/min.
Helium: 10 PSI

GC conditions:

DB-1 fused silica capillary column, 30 meter, 1-µm film thickness, 0.25-mm I.D.
Temperature program: Initial 35°C for 4 minutes, ramp to 100°C at 8°/min., then ramp to 300°C at 15°/min, hold 1-5 minutes.
Run time: 27 min.

MSD conditions:

Transfer line: 280°C
Scan 20-300 amus, EI mode
EMV: set at tuning value
Solvent delay: 0 min. for field samples; if a solvent-spiked tube is analyzed, a solvent delay may be necessary to prevent MS shutdown caused by excessive pressure.

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Appendix F

Correcting Diacetyl Concentrations from Air Samples Collected with NIOSH Method 2557

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Correcting Diacetyl Concentrations from Air Samples Collected with NIOSH Method 2557

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Diacetyl (2,3-butanedione), a diketone chemical used to impart a buttery taste in many flavoring mixtures, has been associated with bronchiolitis obliterans in several industrial settings. For workplace evaluations in 2000–2006, National Institute for Occupational Safety and Health (NIOSH) investigators used NIOSH Method 2557, a sampling and analytical method for airborne diacetyl utilizing carbon molecular sieve sorbent tubes. The method was subsequently suspected to progressively underestimate diacetyl concentrations with increasing sampling site humidity. Since underestimation of worker exposure may lead to overestimation of respiratory health risk in quantitative exposure-effect analyses, correction of the diacetyl concentrations previously reported with Method 2557 is essential. We studied the effects of humidity and sample storage duration on recovery of diacetyl from experimental air samples taken from a dynamically generated controlled test atmosphere that allowed control of diacetyl concentration, temperature, relative humidity, sampling duration, and sampling flow rate. Samples were analyzed with Method 2557, and results were compared with theoretical test atmosphere diacetyl concentration. After fitting nonlinear models to the experimental data, we found that absolute humidity, diacetyl concentration, and days of sample storage prior to extraction affected diacetyl recovery as did sampling flow rate to a much smaller extent. We derived a mathematical correction procedure to more accurately estimate historical workplace diacetyl concentration based on laboratory-reported concentrations of diacetyl using Method 2557, and sample site temperature and relative humidity (to calculate absolute humidity), as well as days of sample storage prior to extraction in the laboratory. With this correction procedure, quantitative risk assessment for diacetyl can proceed using corrected exposure levels for air samples previously collected and analyzed using NIOSH Method 2557 for airborne diacetyl.

Keywords correction equation, diacetyl, humidity effect, sample storage effect

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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 or Occupational Safety and Health Administration.

INTRODUCTION

Diacetyl (2,3-butanedione, CAS no. 431–03-8), a diketone chemical used to impart a buttery taste in many flavoring mixtures, has been associated with severe respiratory disease in several different occupational settings, including microwave popcorn manufacturing, flavoring production, and diacetyl manufacturing.[1–3] Laboratory animal studies have documented that diacetyl alone has toxic properties that are similar to the effects of exposure to diacetyl-containing artificial butter flavoring mixtures.[4,5] The Occupational Safety and Health Administration (OSHA) is in the process of rulemaking on occupational exposure to diacetyl.

National Institute for Occupational Safety and Health (NIOSH) researchers developed and published an analytical method, NIOSH Method 2557, to measure airborne diacetyl in the workplace.[6,7] This method specifies air sample collection through carbon molecular sieve (CMS) sorbent tubes, followed by extraction with acetone/methanol (99:1) and analysis by gas chromatography with flame ionization detection (GC/FID) within 7 days of sampling. Subsequent to the use of this sampling method in several workplace investigations, NIOSH researchers found that the method appeared to progressively

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underestimate diacetyl concentrations with increasing sampling site humidity as compared with OSHA Method PV2118.[8] Silica gel is used as the collection medium in the OSHA method.[9] NIOSH Method 2557 should not be used to measure airborne diacetyl in future studies.

We studied the effect of humidity on measured diacetyl air concentrations using NIOSH Method 2557 with the aim of developing a means for mathematically correcting previously obtained measurements of airborne diacetyl. In addition, we investigated sample storage stability over time because we were aware that some previously obtained field samples had been analyzed beyond the method's specified 7-day maximum storage duration.

METHODS

Protocol

The initial objective of our experiments was to determine if sampling site humidity affects diacetyl recovery in air samples and, if so, to develop a mathematical procedure to correct existing diacetyl air sampling data from previous workplace studies for those effects. NIOSH and OSHA investigators conducted a total of 6 weeks of tests during five visits by NIOSH investigators to the OSHA Salt Lake Technical Center (SLTC) laboratory. During the first week of tests, we started to investigate the effect of humidity and sampling flow rate, as well as the homogeneity of diacetyl mixing in the dynamically generated controlled test atmosphere. During the second and third weeks, we investigated effects of temperature, sampling duration, sampling flow rate, and test atmosphere diacetyl concentration on diacetyl recovery.

Based on results of the first 3 weeks of tests, during the following 2-week test period, we ran tests to further evaluate the effect of test atmosphere diacetyl concentration. In addition, during that 2-week test period we studied sample storage stability using a single test atmosphere diacetyl concentration. Based on the sample storage stability results, we further evaluated the test atmosphere diacetyl concentration effect during a final week of tests. Since we found an effect of sample storage duration on diacetyl recovery, which was dependent on both humidity and test atmosphere diacetyl concentration, the primary objective was extended to include this effect in the mathematical correction procedure.

During each of the five visits, we also collected a number of samples using OSHA Method PV2118 (OSHA 1013[10] was used once it became available) to compare with test atmosphere diacetyl concentration.

Test Atmosphere Generation

Test atmospheres of diacetyl were generated at the OSHA SLTC laboratory by pumping an aqueous diacetyl solution (approximately 1 to 100% diacetyl depending on target concentration), using a syringe pump (Series D; Teledyne Isco Inc., Lincoln, Neb.), through a short length of 0.53 mm diameter uncoated fused silica capillary tubing into a vapor generator where it was heated and evaporated into a dilution

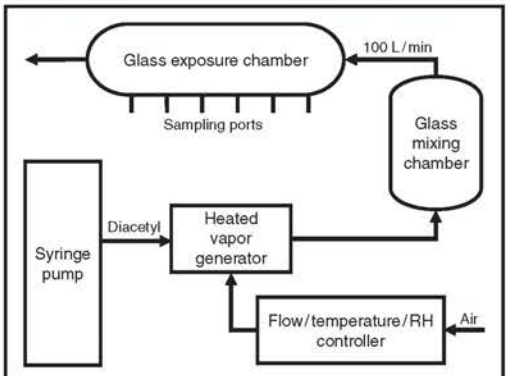

FIGURE 1. Test atmosphere generation apparatus.

airstream (Figure 1). The vapor generator, a 20 cm length of 3 cm diameter glass tubing with a side port for introduction of the capillary tubing, was wrapped with heating tape to evaporate the solution. Humidity, temperature, and volume of the dilution stream of air were regulated by use of a flow-temperature-humidity control system (Model HCS-401; Miller-Nelson Instruments Inc., Pleasanton, Calif.).

The diacetyl-laden air passed from the vapor generator into a glass mixing chamber (76 cm length × 15 cm diameter) and then into a glass exposure chamber (76 cm length × 8 cm diameter). Eighteen evenly spaced glass tube sampling ports extended from the exposure chamber: nine from the bottom and nine from a side. The temperature and relative humidity were measured at the exit of the exposure chamber with a digital thermo-hygrometer (Model RH-411; Omega Engineering, Inc., Stamford, Conn.). The test atmosphere generation apparatus was located in a walk-in hood. Theoretical test atmosphere concentrations of diacetyl were derived using mass flow calculations. These calculations used syringe pump flow rate, chamber airflow rate, and diacetyl concentration in the aqueous solution.

Sampling Procedure

CMS sorbent tubes (Anasorb CMS 226–121; SKC, Eighty Four, Pa.) and pairs (in series) of SKC Model 226–183 silica gel sorbent tubes were attached to the sampling ports, and the test atmosphere was pulled via vacuum through the sorbent tubes with sampling flow rate controlled by adjustable orifices. For each test, flow through each sorbent tube was pre- and post-calibrated with a flowmeter (Model 4100; TSI, Inc., Shoreview, Minn.). After sampling, the sorbent tubes were immediately capped, wrapped in foil, and placed on ice packs in a cooler along with blank sorbent tubes. The coolers were shipped nightly via express mail to a NIOSH-contracted analytical laboratory, where the sorbent tubes were extracted on arrival on Day 1 after sampling (or later, as directed for a few sets of CMS tubes used for storage stability experiments) and analyzed by GC/FID.

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TABLE I. Test Atmosphere Conditions and Sample Numbers

Target Diacetyl Concentration (ppm)	Actual Diacetyl Concentration Mean (ppm)	Actual Diacetyl Concentration Range (ppm)	Actual AH Range (mg H_2O/L air)	Actual RH Range (%)	Actual Temperature Range (°C)	Sampling Duration (hr)	Target Sampling Flow Rate (cc/min)	Number of Samples[A]
Humidity Test Samples								
0.2	0.23	0.23–0.24	4.69–19.12	21–81	23.9–26.2	4, 8	50, 150	87
0.5	0.58	0.56–0.60	3.51–19.26	17–91	22.6–26.3	2, 4, 8	50, 150	107
1.0	1.1	1.1	6.99–14.92	29–62	25.8–26.0	2	50, 150	41
5.0	5.5	5.0–5.9	3.65–22.50	17–92	22.8–27.0, 31.9–33.8	2, 4, 8	50, 150	373
25	24.8	24.5–25.7	3.57–19.06	16–92	22.4–26.1	2	50, 150	109
Stability Test Samples[B]								
0.5	0.57	0.57–0.58	3.51–18.17	17–91	22.6–23.3	4, 8	50	54
5.0	5.6	5.6–5.7	3.65–18.67	17–92	22.8–25.7	2	50	107
25	25.0	24.9–25.1	3.57–18.66	18–92	22.4–22.8	2	50	53

[A]Number of samples used in equation development analyses.
[B]Nine each of the 0.5 and 25 ppm samples and 18 of the 5.0 ppm samples were used in both humidity and storage stability analyses.

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Sampling Test Conditions

Samples were collected between January 2008 and December 2009 during four 1-week periods and one 2-week period of tests. We collected a total of 964 CMS tube samples during 80 tests, with relative humidity (RH) levels ranging from 16 to 92% and temperatures of 22.4 to 33.8°C giving absolute humidity (AH) levels ranging from 3.5 to 22.5 mg H_2O/L air and with diacetyl concentrations ranging from 0.23 to 25.7 ppm. Samples were collected over 2, 4, or 8 hr to test for differences in diacetyl recovery due to sampling duration or because of limit of detection (LOD) concerns during tests at low diacetyl concentrations. Samples were collected using sampling flow rates of 50 or 150 cc/min to investigate any effect on diacetyl recovery associated with differences in sampling flow rate. The test atmosphere conditions and sample numbers are summarized in Table I.

Over the five visits, we collected 134 silica gel samples at a flow rate of 50 cc/min during 43 of the 2-hr tests. These samples were collected with an AH range of 3.57 to 22.50 mg H_2O/L air and diacetyl concentrations from 0.56 to 25.7 ppm.

Sample Storage Stability Tests

In total, storage stability of diacetyl both in the sampling tubes (in-tube) and after extraction from the tubes was investigated using 214 samples (Table I). In the first set of experimental conditions, six sets of triplicate samples were collected at 50 cc/min from a 5.7 ppm diacetyl test atmosphere at each of three AH levels: 3.97, 8.59, and 18.67 mg H_2O/L air (RH = 17, 36, and 78%, respectively, at 25.7°C). Samples were sent overnight on ice to the analytical laboratory, where they were extracted and analyzed according to NIOSH Method 2557 for diacetyl 1, 4, 7, 10, 13, and 16 days post-sampling. All samples were stored in a refrigerator until the scheduled day of extraction.

After analysis of the first set of samples on Day 1 post-sampling, the remaining liquid portion (without sorbent material) of each sample was split into two new vials and one stored at room temperature and the other refrigerated. These samples underwent further stability testing via re-analysis 1, 2, 5, and 11 days post-extraction. New septum caps were placed on each vial after each analysis, and freshly prepared standards were used for each re-analysis. To investigate diacetyl concentration effect on storage stability, during the final week of tests, six sets of triplicate samples each were collected from 0.57, 5.6, and 25.0 ppm diacetyl test atmospheres at each of three mean AH levels: 3.6, 8.5, and 18.5 mg H_2O/L air. The samples were extracted and analyzed 1, 4, 7, 10, 16, and 35 days post-sampling. When splitting the samples for the extract storage stability tests, equal portions of the sorbent material were placed into the two vials with the liquid to better simulate treatment of field samples as directed in Method 2557. Re-analysis of these samples was completed on Days 2, 5, 13, and 34 post-extraction.

Data Analyses

Statistical analysis was carried out using JMP V.8 software (SAS Institute, Cary, N.C.). We used the nonlinear modeling platform to calculate the parameter coefficients for the correction model. Details of models used in the JMP nonlinear platform are discussed in the Results section. We used analysis of variance modeling to investigate effects of sampling port position, sampling duration, and sampling flow rate on percent diacetyl recovered.

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RESULTS

Of 964 CMS samples collected, 717 were used in humidity effect analyses (extraction Day 1 after sampling), 214 were used in sample storage stability analyses (36 of these were used in both analyses), 42 samples from 1 day of tests were excluded due to excessive analytical laboratory variability (the mean coefficient of variation for that day's tests was 73% as compared with a range of 3% to 23% for other days), 13 were excluded due to greater than 5% changes in sampling flow rate during the tests, 3 were excluded due to errors during sampling, 1 had missing data from the analytical laboratory, 1 outlier (greater than 300% recovery) was excluded, and 9 samples collected at low concentration and high humidity were excluded because of nondetectable diacetyl.

Of the 134 silica gel samples, 121 that had matching CMS sample groups during 39 tests were used in the comparison analyses.

Effects of Sampling Port Position, Sampling Duration, and Sampling Flow Rate

During the first week of tests, homogeneous mixing of diacetyl in the exposure chamber was investigated, and analysis of variance indicated no significant effects of sampling port position on diacetyl recovery. An analysis of variance model using data from the first three laboratory visits (n = 448) with

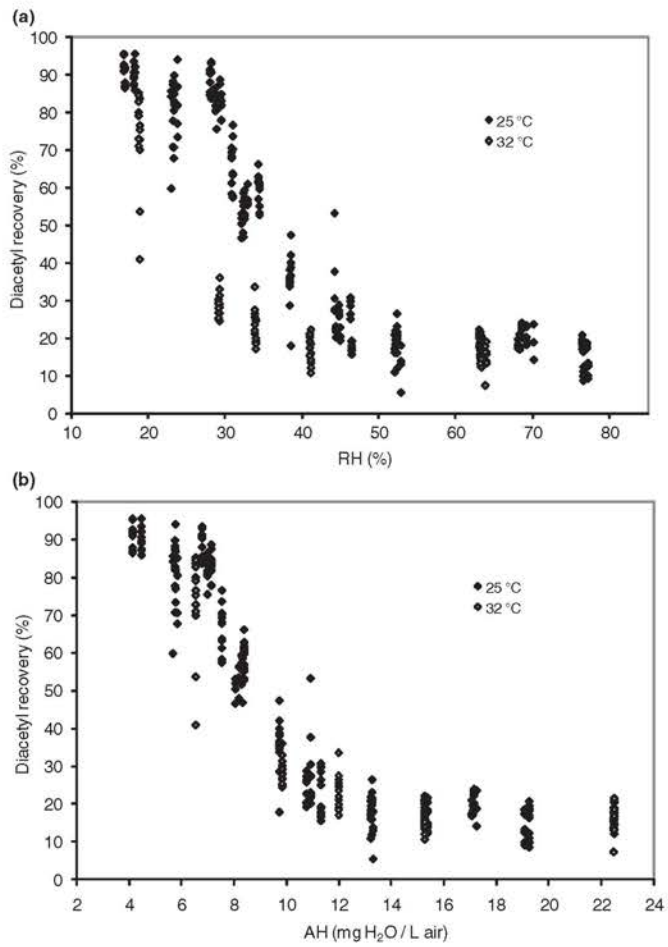

FIGURE 2. Plots for 5.0 ppm target diacetyl concentration data from 25°C and 32°C showing diacetyl recovery in terms of (a) RH and (b) AH.

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percent diacetyl recovery as the outcome variable and AH, test atmosphere diacetyl concentration, target sampling flow rate, and sampling duration as the predictor variables indicated a significant ($p = 0.0042$) effect of sampling flow rate, with percent diacetyl recovery being higher for the 150 cc/min sampling flow rate than for 50 cc/min. The magnitude of the effect was not large; the adjusted means (least squares means) for 150 cc/min and 50 cc/min were 44.9 and 40.3% diacetyl recovery, respectively. In this model there was no significant effect for sampling duration ($p = 0.89$), with adjusted means of 42.3, 41.8, and 43.7% diacetyl recovery for sampling durations of 2, 4, and 8 hr, respectively.

Absolute Humidity Effect—Model for Data from Samples Extracted on Day 1 After Sampling

We investigated the effect of temperature on diacetyl recovery by plotting percent diacetyl recovered against either RH (Figure 2a) in % or AH (Figure 2b) in mg H_2O/L air using data from samples collected from a target diacetyl concentration of 5 ppm at target temperatures of 25°C and 32°C. We calculated AH from RH and temperature (T_c) using Eq. 1, which we derived from a National Weather Service approximation for humidity calculations in surface observations.[11]

$$AH = \frac{13.25\,RH \exp\left(\frac{17.67\,T_c}{T_c + 243.50}\right)}{T_c + 273.15} \quad (1)$$

As seen in Figure 2, the substantial difference in diacetyl recovery for the two temperatures was removed when humidity was expressed as AH. Thereafter, we modeled the percent recovered diacetyl in terms of AH.

Using the JMP model library of nonlinear functions, we visually determined that the 4-parameter logistic function was suitable to describe the sigmoidal relationship of percent recovered diacetyl with humidity, for samples extracted on Day 1 after sampling. The 4-parameter logistic model has parameters θ_1, θ_2, θ_3, and θ_4, each of which has graphical meaning. The parameter θ_1 represents the horizontal asymptote on the right-hand side of the graph where humidity is at the highest level; θ_2 represents the horizontal asymptote on the left-hand side of the graph where humidity is at the lowest level; θ_3 is the "slope" or the shape parameter; and θ_4 is the humidity at which 50% of the maximal response is observed. The general equation in terms of the 4-parameter logistic model is:

$$Y = \theta_1 + \frac{\theta_2 - \theta_1}{1 + \exp[\theta_3(X - \theta_4)]} \quad (2)$$

In our models, percent recovered diacetyl was the Y variable, and humidity was the X variable.

We fitted separate 4-parameter logistic models to the data for each of the target test atmosphere diacetyl concentrations (0.2, 0.5, 5.0, and 20 ppm). We had too few levels of AH for the 1.0 ppm test atmosphere diacetyl concentration to adequately fit the 4-parameter logistic model. Figure 3 shows the separate 4-parameter logistic models for percent recovered diacetyl vs. AH as fitted through the overall test data (for both sampling flow rates combined).

Using information from these models, we created one nonlinear model for the data overall; this model took into account differences in the 4-parameter values for the individual logistic models. We found that θ_1 was well approximated ($R^2 = 0.99$) by a linear function of target concentration C_0

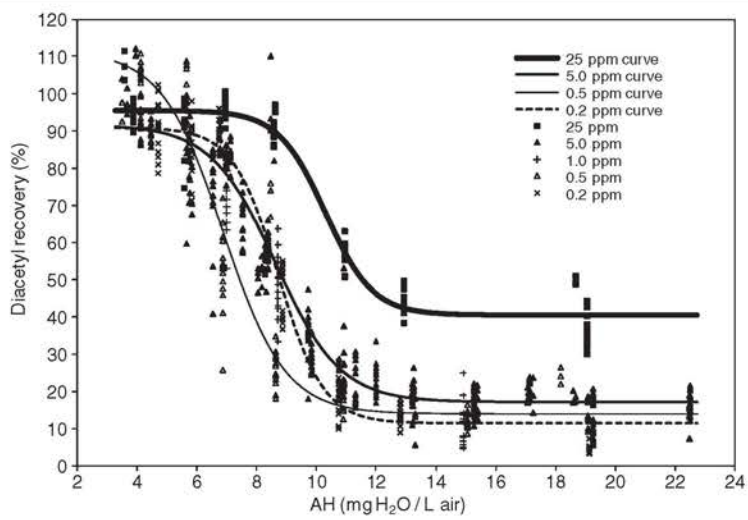

FIGURE 3. Four-parameter logistic models fitted to test data. Note: Data are insufficient to create a logistic curve for 1.0 ppm.

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TABLE II. Parameter Coefficients for the Overall Diacetyl Correction Equation and for Two Sampling Flow Rates

Parameter	Overall	Sampling Flow Rate 50 cc/min	Sampling Flow Rate 150 cc/min
b_0	6.91166	5.85971	8.32618
m_1	1.69272	1.70372	1.68917
θ_2	101.31390	101.06329	101.81000
θ_3	0.72068	0.70943	0.73539
θ_4	8.22607	8.18808	8.26746
q	0.05362	0.05362	0.05362
r	0.41384	0.41384	0.41384
s	–0.00589	–0.00589	–0.00589
u	–0.01719	–0.01719	–0.01719
v	0.30359	0.30359	0.30359
w	0.00558	0.00558	0.00558
x	0.00153	0.00153	0.00153
y	0.26802	0.26802	0.26802
z	–0.00002	–0.00002	–0.00002

(i.e., $\theta_1 = b_0 + m_1 C_0$, where b_0 is the intercept and m_1 is the slope) and so substituted this linear function into the 4-parameter logistic function using the values for b_0 and m_1 as starting values for the overall model. Since the other parameters showed variability but no trend with levels of C_0, we used the arithmetic means of the separate model θ_2, θ_3, and θ_4 parameters for the four target test atmosphere diacetyl concentrations as the overall model starting values for these three parameters. We expressed percent recovered diacetyl ($100c/C_0$, where c is the recovered concentration reported by the laboratory) by rewriting Eq. 2 as follows:

$$\text{Percent recovered diacetyl} = \frac{100c}{C_0} = h(C_0, AH) = b_0 + m_1 C_0 + \frac{\theta_2 - b_0 - m_1 C_0}{1 + \exp[\theta_3(AH - \theta_4)]} \quad \textbf{(3)}$$

We entered this form of the equation (Eq. 3) into the nonlinear fitting platform for a fit through all the data (including the data for a test atmosphere diacetyl concentration of 1.0 ppm). We repeated the fit through the data stratified by sampling flow rate. The final values for the parameters (b_0, m_1, θ_2, θ_3, and θ_4) both overall and for the two sampling flow rates are given in Table II (the table also contains parameter values for the effect of in-tube storage as described below). The R^2 (amount of total variability in the data accounted for by the model) for the overall model was 0.91. The R^2 for the 150 cc/min model was 0.93, and the R^2 for the 50 cc/min model was 0.90. Figure 4 shows how Eq. 3 describes the pattern of diacetyl recoveries for a range of concentrations both overall and for the two sampling flow rates and illustrates that the effect of sampling flow rate was not large.

Equation 3 predicts that at a concentration of approximately 56 ppm, the diacetyl recovery would be approximately 100% at all AH values (this was similar for the overall model and the 50 and 150 cc/min models). At diacetyl concentrations above these values, the model predicts diacetyl recoveries of higher than 100% across the range of AH values, which does not represent a real-life solution. Predicted diacetyl recoveries

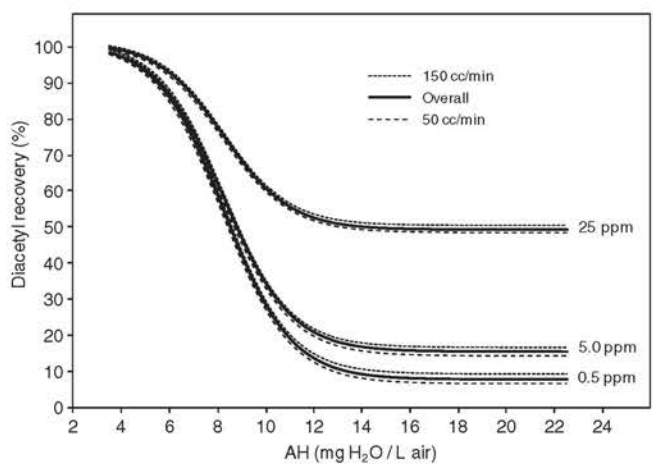

FIGURE 4. Model for data from samples extracted on Day 1 after sampling (Eq. 3) applied at three diacetyl concentrations using parameter coefficients (b_0, m_1, θ_2, θ_3, θ_4) for the whole data set (overall) and the two sampling flow rates used in the experiment.

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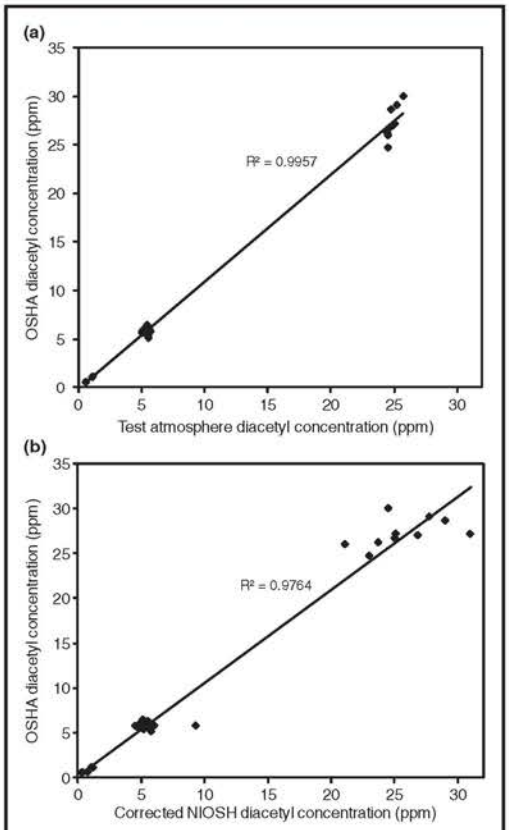

FIGURE 5. Comparison of diacetyl sampling results using OSHA method (a) to calculated test atmosphere concentration, and (b) to corrected values of the diacetyl sampling results using NIOSH Method 2557.

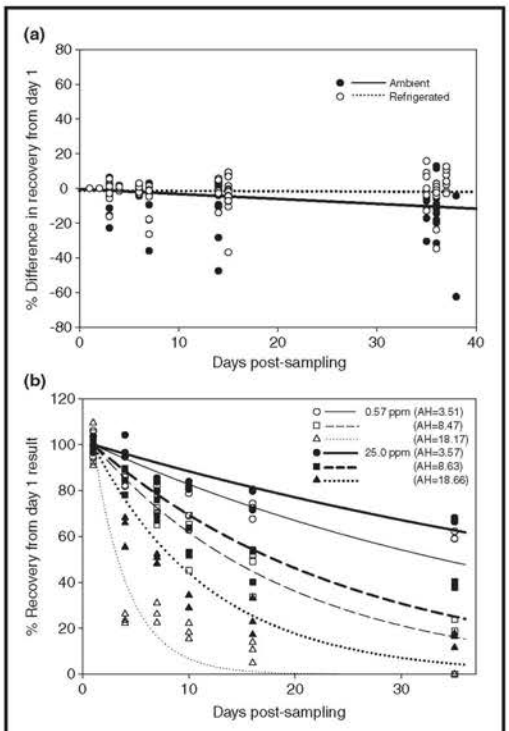

FIGURE 6. (a) Extract stability: Samples extracted and analyzed on Day 1 post-sampling and the remaining sorbent material and extract kept at ambient or refrigerated temperature before further analyses on subsequent days. Lines represent regressions. (b) In-tube stability: Samples extracted and analyzed on the same day. Curves were created from Eq. 4.

from Eq. 3 for very low diacetyl concentrations do not have the same problem since, mathematically, in the limit as the diacetyl concentration goes to zero, the recoveries range from approximately 100% to approximately 7% as AH goes from low to high.

OSHA Silica Gel Sample Results

Diacetyl concentrations from the 121 silica gel samples taken at a number of AH conditions were quite similar to the calculated test atmosphere concentrations (Figure 5a) and were not affected by AH. Using the model (Eq. 3) we calculated the corrected diacetyl concentrations from the matched NIOSH Method 2557 CMS samples, and as shown in Figure 5b, we found a strong linear relationship with the silica gel results.

Model for Effect of In-Tube Storage

Plots of extract storage and in-tube storage stabilities are shown in Figure 6. Samples stored as extracts, either with or without sorbent material, under refrigerated conditions were stable, having less than 2% loss at each of the three AH levels over nearly 40 days of storage (1.4% at 7 days and 1.7% at 38 days). Under ambient conditions, the loss was 2.9% at 7 days and 11.0% at 38 days. In contrast, plots of diacetyl recovered by number of days of in-tube storage (i.e., days from sampling to extraction) indicated decreased recovery over time, with the changes over time showing dependence on both AH and diacetyl concentration. For a given diacetyl concentration, diacetyl losses over time were greater with increasing AH. For a given AH, diacetyl losses over time were greater with decreasing concentration.

To model in-tube storage effects, we used first-order decay functions to estimate decay constants for the 12 combinations of diacetyl concentrations and AH. We normalized the diacetyl recovery data by dividing the diacetyl recovery data by the

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mean for recovery on Day 1 after sampling and included (t-1) in the first-order decay functions (see below). The first-order decay model is given by: Y = (starting amount) exp[-k(t-1)], where starting amount = 1 for normalized data, t = days from sampling to extraction, and k is the decay constant.

We substituted functions of AH and diacetyl concentration for the decay constants (k). This was accomplished in two steps. In Step 1, we fitted quadratic functions to the k values for the three target diacetyl concentration (0.5 ppm, 5 ppm, and 25 ppm) curves of k vs. AH. In Step 2, we substituted three-parameter first-order decay functions for the coefficients for the intercept, the AH term and the AH^2 term of the quadratic function based on the diacetyl concentrations. This gave estimates for the nine coefficients (q, r, s, u, v, w, x, y, and z) in the model (as shown below). In a final step, the values of the nine parameters were used as starting values to get a fit of this nonlinear model through the full set of in-tube storage data. The R^2 for this model was 0.90. The coefficients are given in Table II. The form of the nonlinear model for the effect of in-tube storage was:

$$\text{Normalized recovery} = g(C_0, AH, t) = \exp[-(f_1(C_0) + f_2(C_0)AH + f_3(C_0)AH^2)(t-1)] \quad (4)$$

where

$$f_1(C_0) = q\exp(-rC_0) + s$$
$$f_2(C_0) = u\exp(-vC_0) + w$$
$$f_3(C_0) = x\exp(-yC_0) + z$$

Full Model

The full model can be conceptualized in two steps. First, the AH, the recovered diacetyl concentration (c), and the number of days from sampling to extraction (t) are used to predict the recovered diacetyl concentration on Day 1 of extraction after sampling. Second, this predicted diacetyl value and AH is used to predict the corrected concentration. The full model for the percent of diacetyl recovered is:

$$\text{Percent recovered diacetyl} = \frac{100c}{C_0} = h(C_0, AH)g(C_0, AH, t) \quad (5)$$

where h is given by Eq. 3 and g is given by Eq. 4.

Since, in practice, the values of c, AH, and t are known, and the value of C_0 is the predicted corrected diacetyl concentration, we solved Eq. 5 for C_0. Using Eqs. 3 and 4, Eq. 5 can be rewritten as:

$$aC_0^2 + bC_0 - \left(\frac{c}{g(C_0, AH, t)}\right) = 0 \quad (6)$$

where

$$a = m_1/100 + \frac{-m_1/100}{1+\exp[\theta_3(AH-\theta_4)]}$$
$$b = b_0/100 + \frac{(\theta_2 - b_0)/100}{1+\exp[\theta_3(AH-\theta_4)]}$$

Since this is a nonlinear equation for C_0, it is necessary to use an iterative procedure to find C_0. Initially, Eq. 6 is solved for C_0 using the quadratic formula with the dependence of g on C_0 ignored:

$$C_0 = \frac{-b + \sqrt{b^2 + 4a\left(\frac{c}{g(C_0, AH, t)}\right)}}{2a} \quad (7)$$

(Note: The other solution for Eq. 6 using the quadratic formula yields a nonphysical negative value for C_0 since $a > 0$ and $b > 0$.)

In Eqs. 6 and 7 the value of $\left(\frac{c}{g(C_0, AH, t)}\right)$ is the estimate for the diacetyl concentration corrected for days to extraction after sampling.

To solve Eq. 7, an iterative procedure is used with the i value $C_0^{(i)}$ used to calculate the (i+1) value $C_0^{(i+1)}$

$$C_0^{(i+1)} = \frac{-b + \sqrt{b^2 + 4a\left(\frac{c}{g(C_0^{(i)}, AH, t)}\right)}}{2a} \quad (8)$$

It is necessary to start the procedure with an initial C_0 (i.e., $C_0^{(1)}$). We found the procedure robust to the choice of starting value and suggest the use of c (the recovered concentration reported by the laboratory).

The sequence of solutions is then calculated until two consecutive values for C_0 are identical to a chosen number of decimal places (convergence). We tested the model for regions of convergence using theoretical recovered concentrations (c) from 0.001 to 70 ppm, AH from 2 to 25 mg H_2O/L air, and days to extraction from 1 to 36. We found that convergence occurred for all concentrations above 1.0 ppm. For lower concentrations, convergence occurred whenever AH was less than 14.5 mg H_2O/L air and days to extraction were fewer than 9. The region of convergence improved from a concentration of 0.001 to 1.0 ppm. At 1.0 ppm, convergence occurred whenever AH was less than 21 mg H_2O / L air and days to extraction were fewer than 17.

As discussed above, for values of $C_0 > 56$ ppm, the Day 1 model does not yield real-life solutions. For these values, only the model for effect of days to extraction should be applied and we predict the concentration of diacetyl for Day 1 of extraction after sampling. If the converged value as calculated above is > 56 ppm, use it as the starting value $C_0^{(1)}$ in an iterative procedure using the equation:

$$C_0^{(i+1)} = \frac{c}{g\left(C_0^{(i)}, AH, t\right)} \quad (9)$$

The sequence of solutions is then calculated until two consecutive values for C_0 are identical to a chosen number of decimal places. Figure 7 is a flow diagram of the correction procedure as described above. When corrected diacetyl concentrations fall between 0.23 and 25.7 ppm, which were the lowest and highest diacetyl test atmosphere concentrations

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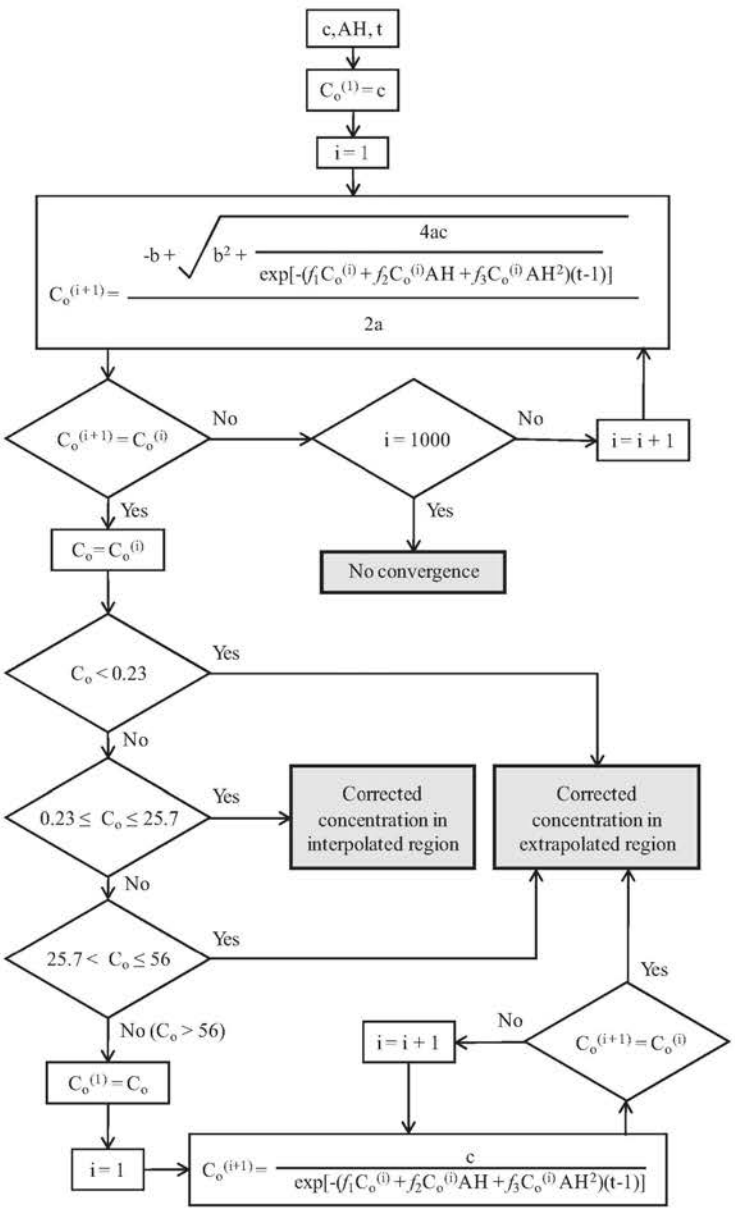

FIGURE 7. Flow diagram of the correction procedure that begins with known values of recovered diacetyl concentration (c), absolute humidity (AH) during sampling, and the number of days from sampling to extraction (t), and ends with the corrected diacetyl concentration in either the interpolated or extrapolated region. As explained in the text, there are some conditions of no convergence.

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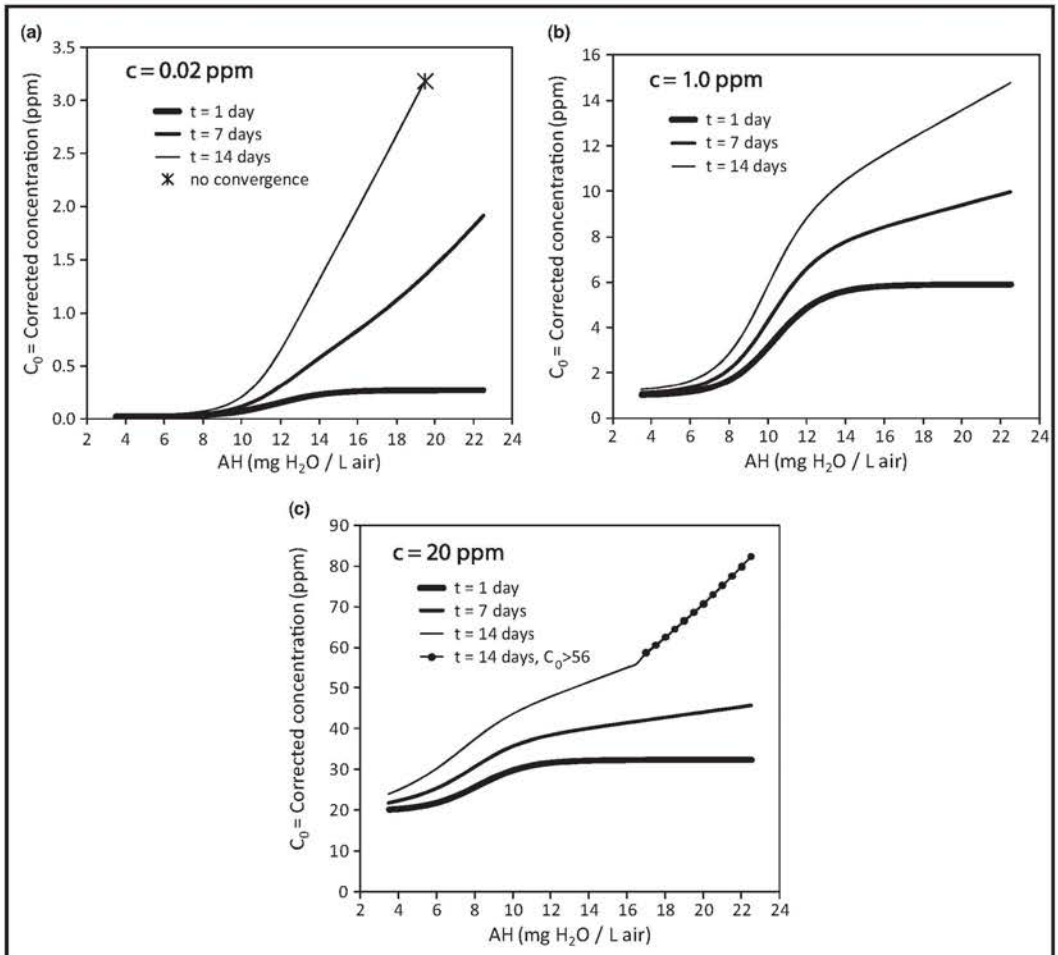

FIGURE 8. Predicted diacetyl concentrations using the model at selected laboratory-reported (recovered) concentrations of (a) 0.02 ppm, (b) 1.0 ppm, and (c) 20 ppm.

used in our experiments, we consider the corrections to be within the interpolated range and have the most confidence in these values. Figure 8 shows diacetyl concentrations predicted by our models for a number of different conditions. We chose three laboratory-reported diacetyl concentrations (c) of 0.02, 1.0, and 20 ppm over a wide range of AH and days from sampling to extraction (t) that should represent possible field conditions. We see that both changes in AH and t substantially affect the value of the corrected concentration. In Figure 8a, we indicate a point where nonconvergence begins for $c = 0.02$ ppm and $AH = 19$ on the 14 days from sampling to extraction curve. In Figure 8c, we indicate a region where $C_0 > 56$, which is only corrected for days from sampling to extraction.

DISCUSSION

From experimental test atmosphere work, we have created a procedure that allows historical diacetyl concentration data from analysis of samples using NIOSH Method 2557 to be corrected to more accurately estimate historical workplace airborne diacetyl concentrations. This correction procedure provides a means for applying these diacetyl concentration estimates in planned quantitative risk assessment relating health effects observed among workers to their diacetyl exposure. In addition, it will allow for a better understanding of historical workplace concentrations of diacetyl that will give insight for exposure control strategies. Use of this correction procedure

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requires laboratory-reported concentrations of diacetyl in ppm (samples collected and analyzed using NIOSH Method 2557), temperature and RH (to calculate AH) conditions at the time of sampling, and the number of days from sample collection to sample extraction for analysis. We give overall parameter values for the full model, as well as for sampling flow rates of 50 and 150 cc/min (Table II). Since the effect of sampling flow rate was not large, investigators have the option of using the overall parameter values, especially if their historical data were collected at sampling flow rates other than 50 and 150 cc/min.

A strength of this work was the use of a controlled test atmosphere to simulate historical field survey conditions where airborne diacetyl was sampled together with humid air. By using two target temperatures with similar ranges of RH, we were able to show that both temperature and RH had an effect on diacetyl recovery and that using AH (mg H_2O/L air) was the key variable to connect the correlation between temperature and concentration. This finding extends the work of McKernan and colleagues,[8] who were unable to separate the effect of temperature and RH in their field-based work. By running tests with several different test atmosphere diacetyl concentrations over a wide range from 0.23 to 25.7 ppm, we were able to observe differences in diacetyl recovery related to theoretical diacetyl concentration. We found a large difference in diacetyl recovery between the test atmosphere diacetyl concentration of about 25 ppm and all the lower concentrations, especially at the higher AH values. The final correction equation predicts that humidity would no longer have an effect on diacetyl recovery at approximately 56 ppm, but we have no empirical data to test this prediction. Corrected diacetyl concentrations that lie outside our test atmosphere range represent extrapolations of the models, and we have less confidence in these concentrations.

We do not suggest the use of the correction procedure with historical concentration data below the limit of detection (LOD), for which concentration may have been estimated (e.g., using LOD/2 or LOD/$\sqrt{2}$). It is not possible to know if the workplace diacetyl concentration was indeed below the LOD or if the losses due to humidity and days from sampling to extraction in the laboratory caused the sample value to be below the LOD. We did find some regions of AH and days from sampling to extraction for recovered concentrations of 1.0 ppm or less where the full model does not converge; however, such conditions should not occur often in the field.

Our storage stability test findings were contrary to the NIOSH Method 2557 specification of good stability for 7 days from sampling to analysis.[7] This may have been due to the fact that storage stability tests completed during method development used spiked sampling tubes without using humid air rather than our actively sampled tubes using a test atmosphere. As our results showed, early extraction minimized further sample loss, especially when the samples were refrigerated in accordance with the method, which means that delays in analysis after extraction should not cause appreciable loss. A limitation of our work is that we did not collect in-tube storage data for all the tests to determine the effect of AH on sample recovery, but we did collect data for three target test atmosphere diacetyl concentrations and three target AH values. Thus, we estimated the effect of AH and the effect of in-tube storage on different data sets and combined the two models mathematically to create the final model.

Our correction equations accounted for about 90% of the variability in the experimental data by taking into account the effects of AH, test atmosphere diacetyl concentration, sampling flow rate, and days of in-tube storage. The variability seen in the data at any combination of AH and diacetyl concentration values has a number of sources, including variability in keeping test atmosphere conditions constant, variability in sampling flow rates during the tests, sampling duration differences (although not found significant), and analytical laboratory variability.

Our test atmosphere experiments used no flavoring chemicals besides diacetyl. In field situations, diacetyl may occur together with other chemicals in the air. Any effect of these mixtures on the diacetyl recovery using NIOSH Method 2557 might not be accounted for with our correction procedure.

Comparison between corrected diacetyl concentrations and the results from side-by-side samples taken with OSHA methods indicated a high correlation, which increases our confidence in the applicability of the correction method. Despite the limitations, the correction procedure enables more accurate quantitative risk assessment now under way for regulatory guidance on occupational exposure to diacetyl. Representative exposures in the flavoring manufacturing industry are difficult to assess because of short-duration batch production methods in which hour-to-hour and day-to-day variations in diacetyl exposures is expected in workplaces where scores of different kinds of flavorings are manufactured. Hence, relative stability of diacetyl exposures in microwave popcorn production facilities offers the advantage of less potential for exposure misclassification.

However, without appropriate correction, the systematic underestimation of true diacetyl exposures in the 2000–2006 historical data would lead to overestimation of health risk associated with diacetyl exposure. Accordingly, use of our correction procedure to recalculate the historical exposure estimates from microwave popcorn production facilities previously studied by NIOSH and others will contribute to ongoing efforts to understand the health risk associated with occupational exposure to diacetyl. Our experimental work may also motivate further research exploring the mechanism by which analyte recovery from CMS sorbent may be affected by sampling site humidity for a variety of analytes.

CONCLUSIONS

We have developed a mathematical procedure that allows measurements from historical diacetyl samples collected and analyzed using NIOSH Method 2557, which

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may be biased low, to be adjusted for a more accurate exposure assessment. In addition to the historical laboratory-reported diacetyl concentrations, this correction procedure requires data on AH (determined from temperature and RH measurements) during sampling and on the number of days between sample collection and laboratory extraction of the sampling tubes. NIOSH Method 2557 should not be used to measure airborne diacetyl in future studies.

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Appendix G

JEM Tables for Four Plants

JEM Tables for Four Plants

The mean diacetyl vapor concentrations estimated for the three plants with a single NIOSH survey are shown in Tables A3.1 – A3.3. For the fourth plant, Company G, time-dependent exposure levels were estimated in the NIOSH-OSHA JEM collaboration (Table A3.4). All Method 2557 measurements were corrected for temperature, humidity, and days to extraction.

Table A3.1 Diacetyl exposure matrix for health hazard evaluation: Company N

Department	Job	n	ppm diacetyl
Maintenance office	Maintenance / mechanic	2	0.164
Microwave popcorn line	Bag placer	2	0.696
	Machine operator	3	1.160
	Packer / Stacker	2	0.143
	(area)	2	0.621
Mixing, measuring room	Tank mixer	1	0.794
	(area)	2	1.032
Packing area	Packer / Stacker	2	0.121
	(area)	2	0.159
Poly line	Poly line worker	2	0.235
	(area)	2	0.182
Quality control area	Quality control worker	2	0.250
	(area)	2	0.320
Stencil area	Stenciler	2	0.045
	(area)	2	0.024
Warehouse	Fork lift operator	2	0.005

Table A3.2 Diacetyl exposure matrix for health hazard evaluation: Company K

Department	Job	N	ppm diacetyl
41-A Entire Area	Micro Pdn—Manager/Supvr	1	0.003
	Micro Pdn—Sanitation/Cleaning	1	0.003
41-A Blending Room (Mixing)	Micro Production—Mixer	7	0.913
41-A Carton / Tray	41-A Filler Side	1	0.002

	Micro Pdn—Maint/Mechanic	1	0.054
	Micro Pdn—Production Worker	5	0.039
41-A Case / Pallet	Micro Production—Stacker	5	0.002
41-A Filler Side	41-A Case/Pallet	8	0.038
	Plant—Maintenance/Mechanic	1	0.003
	Plant—Other	1	0.002
41-A Lab	41-B Warehouse	5	0.003
41 Warehouse	Plant—Other	1	0.268
41-B Warehouse	Micro Pdn—Forklift Operator	1	0.002
	Plant—Other	1	0.002
41 & 41-B Warehouses	Micro Production—Stacker	1	0.002
41-Microwave Pdn Building	Micro Pdn—Maint/Mechanic	2	0.037
	Micro Pdn—Manager/Supvr	3	0.003
	Micro Production—Mixer	1	0.002
	41-B Warehouse	1	0.003
	Micro Pdn—Production Worker	1	0.002
	Plant—Other	1	0.003
Office Building	Office/Management/Sales	2	0.002
Poly Production Building	Poly—Production	2	0.309
	Poly—QC	2	0.002
Ambient	Ambient	2	0.003
Pre-mix Area	Micro Production—Mixer	3	0.043

Table A3.3 Diacetyl exposure matrix for health hazard evaluation: Company L

Department	Job	N	ppm diacetyl
Press Room	Press room worker	10	0.015
Slurry Room (Mixing)	Supervisor	3	0.723
	Mixer	13	1.426
Packaging	Supervisor	8	0.020
	Phaser operator	10	0.026
	Case packer operator	10	0.026
	Cartoner operator	13	0.031
	Palletizer operator	9	0.033
	Line sanitation	3	0.024
	Forklift operator	6	0.036
QA	QA monitor	11	0.025
Warehouse	Supervisor	1	0.033
	Warehouse worker	7	0.035
	Forklift operator	1	0.037
Main Office	Office worker	6	0.025
Maintenance	Press room worker	1	0.011
	Supervisor	1	0.012
	All over plant	2	0.006
	Other	5	0.021
Ambient	Outside	1	0.003

Table A3.4 Diacetyl exposure matrix for health hazard evaluation: Company G

Department	Job	Period	ppm diacetyl	Start Date	End Date
Microwave Production	Oil mixing	1	9.713	7/1/1986	2/11/2001
Mixing room		2	2.509	2/12/2001	4/5/2001
		3	0.245	4/6/2001	9/6/2002
		4	0.006	9/7/2002	8/15/2003
Office	Office	1	0.009	7/1/1986	8/15/2003
Office	Lab technician/quality control	1	0.335	7/1/1986	2/11/2001
		2	0.250	2/12/2001	5/21/2001
		3	0.123	5/22/2001	8/8/2002
		4	0.034	8/8/2002	3/8/2003

4

		5	0.007	3/9/2003	8/15/2003
Warehouse	Warehouse	1	0.557	7/1/1986	2/11/2001
		2	0.017	2/12/2001	8/15/2003
Warehouse/Microwave	Bag checker and bag machine operator	1	1.613	7/1/1986	2/11/2001
Production		2	0.947	2/12/2001	5/21/2001
		3	0.053	5/22/2001	9/6/2002
		4	0.003	9/7/2002	8/15/2003
Polypropylene	Supervisor, machine operator, line packer,	1	0.047	7/1/1986	2/11/2001
	line stacker	2	0.020	2/12/2001	8/15/2003
Microwave Production	Maintenance	1	1.145	7/1/1986	2/11/2001
and All Over		2	0.294	2/12/2001	5/21/2001
		3	0.054	5/22/2001	9/6/2002
		4	0.002	9/7/2002	8/15/2003
Microwave Production	Supervisor, machine opr, do-boy opr,line	1	2.668	7/1/1986	2/11/2001
	packer, line stacker, inventory control,	2	0.672	2/12/2001	5/21/2001
	fill-in on line, box folder	3	0.343	5/22/2001	9/6/2002
		4	0.003	9/7/2002	8/15/2003
Microwave Production	Lab technician/quality control	1	1.312	7/1/1986	2/11/2001
Quality Control Lab		2	0.974	2/12/2001	5/21/2001
		3	0.467	5/22/2001	8/8/2002
		4	0.108	8/8/2002	3/8/2003
		5	0.002	3/9/2003	8/15/2003
Microwave Production	Line packer/machine operator/mixer	1	5.016	7/1/1986	2/11/2001
		2	1.284	2/12/2001	4/5/2001
		3	0.530	4/6/2001	5/21/2001
		4	0.311	5/22/2001	9/6/2002
		5	0.004	9/7/2002	8/15/2003
Outside / Yard	Maintenance and outside processing	1	0.009	7/1/1986	8/15/2003
All Over / Float	Supervisor and janitor	1	2.068	7/1/1986	2/11/2001
		2	0.685	2/12/2001	4/5/2001
		3	0.402	4/6/2001	5/21/2001
		4	0.165	5/22/2001	8/8/2002
		5	0.105	8/9/2002	9/6/2002
		6	0.026	9/7/2002	3/8/2003
		7	0.009	3/9/2003	8/15/2003
All Over / Float	Exterminator	1	0.009	7/1/1986	8/15/2003
Microwave and	Line packer, line stacker on both lines	1	1.358	7/1/1986	2/11/2001
Polypropylene		2	0.346	2/12/2001	5/21/2001

Production				
	3	0.182	5/22/2001	9/6/2002
	4	0.012	9/7/2002	8/15/2003

6

Appendix H

Development of a Job Exposure Matrix for Company G

1.0 Development of a Job Exposure Matrix for Company G

1.1 Overview

To estimate worker exposures for risk assessment, we developed a job exposure matrix (JEM) containing estimates of the average 8-hour, time-weighted average (TWA) exposure levels for diacetyl vapor in parts per million parts air (ppm). This JEM includes estimates for eight major job categories with selected time periods specific for each job category to reflect changes in processes and engineering controls over time. The exposure levels presented in the JEM are based on diacetyl air sampling data collected during nine industrial hygiene surveys conducted by NIOSH industrial hygienists between November 2000 and July 2003 at a microwave popcorn plant in Missouri [Kreiss et al. 2002; Kullman et al. 2005; NIOSH 2006]. Details of the JEM construction are described below.

1.2 Industrial Hygiene Surveys

A total of nine industrial hygiene surveys were conducted over a period of 4 years from 2000 to 2003. The sampling was typically conducted during the day shift, because this shift presented the opportunity to sample all job categories. However, samples were also collected from second and third shifts, but not routinely. The dates for these industrial hygiene surveys are presented in Table A4.1.

Personal breathing zone (PBZ) and area diacetyl samples were collected during these surveys using NIOSH Method 2557. These measurements were subsequently adjusted to account for interferences due to humidity and sample storage [Cox-Ganser et al. 2011]. During all surveys except the first, full-shift PBZ samples were collected from workers performing typical tasks representative of each of the major job categories. In addition, concurrent full-shift area samples were taken throughout the plant from locations where workers would typically spend their time. The PBZ sample measurements were used to develop the exposure estimates for the eight job categories in the JEM. In some instances where personal diacetyl samples were not collected, for example during the first survey, area samples were used to obtain estimates of personal-equivalent diacetyl exposures.

1.3 Creation of Job Categories and Estimation of Arithmetic Means

For the purpose of developing exposure estimates for the JEM, plant job titles were aggregated into eight job categories based primarily on work and environmental similarities with respect to potential for diacetyl exposures [Corn and Esmen 1979]. These eight categories are listed in Table A4.2 along with the jobs that comprise each category.

Arithmetic means (AM) using PBZ samples were calculated for the cells in the JEM as the AMs are the preferred measure of central tendency for estimating cumulative exposure in chronic disease investigations [Smith 1992]. Few PBZ measurements were collected for most job categories in each of the nine surveys (range: n=1–6) except for the job category of Microwave line (range n=11–18). Moreover, a large fraction of the PBZ measurements were below the limit of detection (LOD) for most job categories (>50%) especially during surveys 6–9, except for the job categories of microwave packaging line, quality control and microwave mixing. Thus because of the small sample size and large fractions of LOD data, a simple substitution method

of replacing LOD measurements with a value of LOD/2 was used [Ganser and Hewett 2010]. The calculation of the arithmetic mean exposures by the different time periods is described in detail in the next section on "Creation of Exposure Periods."

As noted earlier, PBZ diacetyl samples were not collected during survey 1 and had to be estimated from personal and area samples collected during subsequent surveys (i.e. surveys 2-9). A hierarchical approach was used to estimate the PBZ exposures for survey 1 depending on the job category and the availability and fraction of personal or area measurements below the LOD. To start with, for jobs categories with sufficient personal and area samples in surveys 2–9, a prediction model was used to estimate personal exposures from area exposure measurements (e.g. microwave mixers, microwave line, quality control). For job categories with small sample size and/or large fraction of measurements below the LOD for surveys 2–9, the arithmetic mean of the area samples from survey 1 was assigned to personal estimates for survey 1, assuming a ratio of 1 for personal to area measurements (e.g., warehouse, outside/office, polyethylene line). For jobs with no area measurements in survey 1 (e.g., bag print) or the area measurements were not representative of personal measurements (maintenance), exposure estimates from similar jobs in survey 1 were assigned. The detailed approach to calculate personal-equivalent diacetyl exposure for each job category for the first survey is described in Table A4.3.

1.4 Creation of Exposure Periods

After estimating the personal-equivalent exposures for survey 1, arithmetic means were calculated for the different time periods. Unique exposure time periods were developed for each of the eight job categories to reflect impact of the exposure control changes implemented at the plant between November 2000 to July 2003. Table A4.4 lists these exposure control changes according to the time of implementation. These exposure time periods varied by job categories since some control changes would have a greater impact on some job categories than others. In addition, the fraction of LOD samples for job categories during the different surveys also impacted the creation of time periods. Surveys for which a large fraction of the measurements for a job category were below the LOD were combined into one time period. For example, for warehouse, 50%–100% of personal measurements were below the LOD for surveys 3–9, hence these surveys were combined into one time period. For the selection of time periods for the job categories, the LOD patterns were consistent with the implementation of controls. The detailed approach used to create the time periods for each job category is described in Table A4.5. Thus a JEM was created consisting of 8 job categories and 2–5 time periods spanning the time duration from November 2000 to July 2003.

1.5 Adjustment for Respirator Use

The JEM created as described above was based on samples collected from workers breathing zone and did not account for respirator use by workers. However, during survey 4 and onward, workers in microwave mixing were using respirators and the JEM estimates were adjusted in the appropriate time periods to reflect the PPE use. Thus for the mixers during time periods 3 and 4, we adjusted the measured personal diacetyl exposure for the use of respirators. During these time periods, mixers used respiratory protection while in the mixing room and these respirators included either a PAPR or air-line respirator with a loose fitting hood; both types of respirators have an applied protection factor (APF) of 25 [NIOSH 2004]. We assumed, based on survey observations and questionnaire responses, that mixers spent, on average, about 4 hours per shift

in the mixing room in respiratory protection. Because respirators were required in the mixing room by plant management, we assumed that mixers wore respirators at all times while in the mixing room and did not wear these respirators when outside the mixing room and in the microwave packaging room. During these time periods, the mixers' desk was located in the microwave packaging room near packing line 1 so we further assumed that, when not in respirators in the mixing room, mixers would be in the microwave packaging area and receive diacetyl exposures consistent with those personal exposures measured in microwave packaging. The mixer personal samples were taken outside of the respirator and would reflect both mixing and packaging exposure components. Accordingly, to adjust mixer exposures to diacetyl for the use of respirator, we (1) determined the mixing room exposure component from the combined mixing and packing line diacetyl concentration as reflected in the personal sample (back calculated) and (2) applied a protection factor of 25 to the mixing room component of the mixers exposure.

To determine the mixing room (A) personal diacetyl exposure from the combined mixing and packaging (C) concentration measured by personal sampling we applied the following equation:

$C = (4A_{(mixing)} + 4B_{(packaging)})/8$; solving for A gives, $A = 2C - B$
Where A = the mixing room personal exposure component in ppm, B = the packaging room personal exposure component in ppm, and C = the measured mixer personal exposure in ppm reflecting both mixing room and packaging room components.

To correct the mixers exposure for the use of respiratory protection, we used the following equation:

$C_R = ½ (A/25 + B)$ where C_R = respirator adjusted mixer diacetyl exposure in ppm, A= personal mixer diacetyl exposure in the mixing room in ppm and B = personal diacetyl exposure in the microwave packaging area in ppm.

This adjusted diacetyl concentration in ppm was applied to mixers for time periods 3 and 4 to adjust for the use of respiratory protection by mixers while in the mixing room.

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Table A4.1 Industrial Hygiene Survey Dates

Survey	Survey Dates
1	November 11 – 18, 2000
2	Jan 17 – 19, 2001
3	April 1 – 4, 2001
4	September 4 – 8, 2001
5	November 6 – 8, 2001
6	March 18 – 21, 2002
7	August 11 – 16, 2002
8	January 27 – 31, 2003
9	July 14 – 18, 2003

Table A4.2 Exposure Categories used for JEM

#	Exposure Category	Jobs Included in Exposure Category
1	Warehouse	Warehouse Job Category
2	Maintenance	Maintenance Job Category
3	Outside Processing / Office	Outside Processing & Office Job Categories
4	Polyethylene Line	Polyethylene Packer and Polyethylene Stacker Job Categories
5	Microwave Mixing	Microwave Mixer Job Category
6	Microwave Packaging Line	Microwave Job Categories: Machine operator, Packer, Stacker, Supervisor, and Inventory Control
7	Bag Print	Bag Print Job Category
8	Quality Control	Quality Control Job Category

Table A4.3 Procedures Used for Estimating Personal Equivalent Diacetyl Exposures for Survey 1

#	Exposure Category	Procedures used by Exposure Category
1	Warehouse	Use the mean of the area sample diacetyl concentrations from survey 1.
2	Maintenance	Calculate ratio of the survey 3 diacetyl mean for personal samples to the average of survey 3 diacetyl mean from personal samples for polyethylene, mixer, and microwave packaging line job categories. Apply this ratio to the average of the same three groups from survey 1 after they have been converted to personal equivalent exposures.
3	Outside Processing / Office	Use the mean of the area sample diacetyl concentrations from survey 1.
4	Polyethylene Line	Use the mean of the area sample diacetyl concentrations from survey 1.
5	Microwave Mixing	Model personal to area diacetyl concentrations from surveys 2-9 and apply model to survey 1 area samples to determine personal equivalent diacetyl exposures.
6	Microwave Packaging Line	Model personal to area diacetyl concentrations from surveys 2-9 and apply model to survey 1 area samples to estimate personal equivalent exposures.
7	Bag Print	Use the average of the personal equivalent diacetyl exposures for survey 1 from the microwave packaging line and warehouse job categories. (Note: there were no bag print area diacetyl samples for survey 1)
8	Quality Control	Model personal to area diacetyl concentrations from surveys 2-9 and apply model to survey 1 area samples to determine personal equivalent diacetyl exposures.

Table A4.4. Dates of exposure control changes and NIOSH industrial hygiene surveys.

Date	Event
Cross-Sectional Industrial Hygiene Survey, Respiratory protection training by NIOSH (November 11-18, 2000)	
Engineering Control Technology Survey (January 17 – 19, 2001)	
February 12, 2001	Exhaust fan installed in oil and flavoring mixing room
February 2001	Heated liquid flavoring tanks (2) vented to exhaust fan
March 29, 2001	Pump installed for closed transfer of flavorings between holding and mixing tanks
Follow-up Survey (April 2-5, 2001)	
April 6, 2001	Mixers supplied with powered air-purifying respirators and respirator training. Respirators available to workers in other microwave production areas on voluntary basis
May 22, 2001	Local exhaust ventilation installed for 2 of 7 oil tanks on mezzanine. (Note, tanks were initially vented into packaging area air until September 2001)
June 6, 2001	Flavoring storage cabinets completed for storing bulk flavorings
July 16, 2001	Temperature control installed on one flavoring tank
August 7, 2001	Tempered, outside supply air intake system completed, providing replacement air for microwave popcorn production areas
Follow-up Survey (September 4 - 8, 2001)	
September 11, 2001	Exhaust fan installed in quality control lab
September 18, 2001	Fresh air intake installed in quality control lab
September 21-30, 2001	Completion of local exhaust ventilation for all mezzanine oil tanks
November 2, 2001	Flavoring transfer pump installed for 5-gallon containers
November 2, 2001	Air lock installed outside of mixing room
Follow-up Survey (November 6 - 8, 2001)	
Follow-up Survey (March 18 - 21, 2002)	
August 2, 2002	Started use of supplied-air respirators for mixers in mixing room and mezzanine (air-purifying respirators with organic vapor cartridges and particulate filters had been used prior to this.)
August 9, 2002	Microwave ovens and testing counter in quality control lab enclosed with plastic curtain
Follow-up Survey (August 11 - 16, 2002)	
September 7, 2002	Started using new mixing room (ventilation incomplete)
September 30, 2000	Discontinued use of one paste butter flavoring
October 1, 2002	New mixing room wall exhaust fan operational
Follow-up Survey (January 27 - 31, 2003)	
March 9, 2003	Enclosure of tanks on mezzanine completed
April 10, 2003	Air-handler functional on mezzanine
April 15, 2003	New exhaust fan operational in quality control lab (in new "popping room")
April 15, 2003	2 additional exhaust fans (for mezzanine and mix room)

May 13, 2003	Microwave ovens moved into popping room in quality control lab
Follow-up Survey (July 14 - 18, 2003)	

Table A4.5 JEM Exposure Time Periods by Exposure Category

#	Exposure Category Time Periods[1]
1	**Warehouse:** Time 1 (Surveys 1 & 2 sampling results): Reflects exposures before major control changes that would impact warehouse worker exposures. Warehouse workers would go into microwave packaging, primarily on fork-lifts to remove finished product, and could receive higher, packaging area exposures accordingly. Time 2 (Surveys 3 – 9 sampling results): Reflects the control changes implemented in the microwave mixing room in February of 2001 including the addition of exhaust ventilation, closed transfer of liquid flavorings, and flavor tank ventilation. These mixing room control changes impacted warehouse diacetyl exposures since warehouse workers would enter microwave production area daily. Additionally, in August of 2001, the installation of an outside supply air intake system provided clean, tempered air into the warehouse area and makeup air for microwave production axial wall exhaust fans. This allocation of time periods was also selected since a majority of warehouse exposures to diacetyl were non-detectable since April of 2001.
2	**Maintenance:** Time 1 (Surveys 1 & 2 sampling results): Reflects exposures before major control changes that would impact microwave production exposures, including maintenance worker exposures since maintenance workers would work on the production lines as well as in the microwave mixing room. Time 2 (Survey 3 sampling results): Reflects the control changes implemented in the microwave mixing room in February of 2001 including the addition of exhaust ventilation, closed transfer of liquid flavorings, and flavor tank ventilation. These mixing room control changes would affect maintenance worker exposures since they would work in mixing and microwave production and there was a maintenance office located in microwave production. Time 3 (Surveys 4 – 7 sampling results): Reflects the control changes including the installation of an outside supply air intake system providing clean, tempered air into the warehouse, the completion of LEV ventilation on mezzanine flavor holding tanks and the air-lock installation on the mixing room. All these microwave mixing and production control changes would impact maintenance workers since they would work in these production areas. Also, maintenance exposures during this time period were still primarily above the LOD.

	Time 4 (Surveys 8 & 9 sampling results): Reflects the control changes including first use of enclosure of the mezzanine tanks. Additionally, maintenance exposures during this time period were largely below detectable limits.
3	**Outside Processing / Office Workers:** Time 1 (Surveys 1 – 9 sampling results): Reflects low, predominantly non-detectable exposures for workers who were outside (outside processing) or normally away from microwave mixing and production operations.
4	**Polyethylene Line:** Time 1 (Surveys 1 & 2 sampling results): Reflects polyethylene line worker exposures before major control changes in the microwave production area that could impact polyethylene line workers; although exposures in this category were low by comparison to microwave production lines, there were some detectable diacetyl exposures in personal and area samples for polyethylene line workers during this time period so a separate time period was used. While the polyethylene lines were located away from the microwave production area, there was some potential for exposure in this work group prior to control changes. Time 2 (Survey 3-9 sampling results): Reflects the first control changes implemented in the microwave mixing room (including the addition of exhaust ventilation, closed transfer of liquid flavorings, and flavor tank ventilation) plus all subsequent changes. After the first implementation of exposure control in microwave mixing and production areas, exposure among polyethylene line workers were largely below detectable limits.
5	**Microwave Mixers:** Time 1 (Survey 1 & 2 sampling results): Reflects exposures before major control changes that would impact microwave mixer exposures. Time 2 (Survey 3 sampling results): This time period reflects the first control changes implemented in the microwave mixing room including the addition of exhaust ventilation, closed transfer of liquid flavorings, and flavor tank ventilation. During this time, the mixers desk was moved outside of the mixing room as an administrative control. This time period was also before the use of powered air purifying respirators (PAPRs) or air-line respirators. Time 3 (Surveys 4 – 7 sampling results): Reflects significant control changes for mixing workers including first use of PAPR respirators in April of 2001 as well as subsequent use of supplied-air respirators in August of 2002 which would have significantly reduced mixer exposures. Both types of respiratory protection employed loose-fitting hoods with an applied protection factor (APF) of 25. (See description below on procedures used to estimate mixers exposure adjusting for respirator use). Other significant changes during this time period would include the addition of an outside supply- air system which provided clean, tempered make-up air for microwave production and mixing room air

	exhaust fans. Time 4 (Survey 8 & 9 sampling results): Reflects the first use of the new mixing room and the addition of new mixing room exhaust fans. This time period also reflects enclosure of the mezzanine area reducing microwave packaging exposures outside the mixing room below quantifiable or detectable levels; this would affect mixers exposures when in the microwave packaging area and not in respiratory protection in the mixing room.
6	**Microwave Line:** Time 1 (Surveys 1 & 2 sampling results): Reflects exposures before major control changes that would impact microwave line exposures. Time 2 (Survey 3 sampling results): Reflects the first control changes implemented in the microwave mixing room including the addition of exhaust ventilation, closed transfer of liquid flavorings, and flavor tank ventilation which would impact microwave line exposures since the mixing room was adjacent and open to the mixing lines. Time 3 (Surveys 4 – 7 sampling results): Reflects several control changes including the installation of an outside supply air intake system providing clean, tempered air for microwave production area exhaust fans. This period also reflects completion of LEV ventilation on mezzanine flavor holding tanks and air-lock installation isolating the mixing room from packaging areas. Time 4 (Surveys 8 & 9 sampling results): Reflects the first use of the new mixing room and the addition of new mixing room exhaust fans. This period also reflects enclosure of the mezzanine area reducing microwave production exposures outside the mixing room below quantifiable or detectable levels.
7	**Bag Printing:** Time 1 (Survey 1 & 2 sampling results): Reflects exposures before major control changes that would impact bag printing exposures due to close proximity to the microwave production lines. Also, when the bag printing operations were shut down, bag print workers would often work on the microwave production lines. Time 2 (Survey 3 sampling results): Reflects the first control changes implemented in the microwave mixing room including the addition of exhaust ventilation, closed transfer of liquid flavorings, and flavor tank ventilation. These control changes would impact bag printing exposures since the bag print lines were located in the warehouse just outside a large open doorway into microwave production; additionally, when the bag printing operations were shut down, bag print workers would often work on the microwave production lines. Time 3 (Surveys 4 – 7 sampling results): Reflects several control changes including the installation of an outside supply air intake system providing clean, tempered air into the

	warehouse and subsequently for microwave production area exhaust fans. This period also reflects completion of LEV ventilation on mezzanine flavor holding tanks and air-lock installation isolating the mixing room from packaging areas. Time 4 (Surveys 8 & 9 sampling results): Reflects the first use of the new mixing room and the addition of new mixing room exhaust fans. This period also reflects enclosure of the mezzanine area reducing microwave production exposures outside the mixing room below quantifiable or detectable levels.
8	**Quality Control:** Time 1 (Surveys 1 & 2 sampling results): Reflects exposures before major control changes that would impact microwave quality control exposures. Time 2 (Survey 3 sampling results): Reflects the first control changes implemented in the microwave mixing room including the addition of exhaust ventilation, closed transfer of liquid flavorings, and flavor tank ventilation. These changes would impact quality control exposures since the quality control room opened into the microwave production area. Also, the quality control room was generally under negative pressure relative to the microwave production room at this time. Time 3 (Surveys 4 – 6 sampling results): Reflects installation of an exhaust fan and fresh air intake in the quality control lab. It also reflects several changes that would impact quality control worker exposures through reduction of diacetyl concentrations in the microwave mixing and production areas including the installation of an outside supply air intake system providing clean, tempered air into the warehouse and subsequently for microwave production area exhaust fans; installation of a mixing room air-lock; and ventilation of mezzanine flavor holding tanks. Time 4 (Surveys 7 & 8 sampling results): Reflects the enclosure of the microwave ovens in the quality control lab. Time 5 (Survey 9 sampling results): Reflects the relocation of all microwave ovens into a separate, ventilated room. This step reduced quality control exposures below detectable levels. Other control changes to the microwave production area during this period reduced microwave production exposures below detectable levels further impacting quality control exposures.

Appendix I

Typical Protocol for Collecting Air Samples for Diacetyl and 2, 3-Pentanedione

Typical protocol for collecting air samples for diacetyl and 2, 3-pentanedione.

While the elements of a well-designed exposure monitoring program are discussed in Chapter 10, the details of a typical sampling protocol for determination of airborne concentrations of diacetyl and 2, 3-pentanedione vapor are described here. This protocol, which is based on OSHA Method 1012 (Appendix 2 - A), is available at http://www.osha.gov/dts/sltc/methods/validated/1012/1012.html. The same air sampler is specified in OSHA Method 1012 and 1013 for diacetyl, and in OSHA Method 1016 for 2,3-pentanedione. It consists of two silica gel tubes connected in series using the least amount as possible flexible tubing. Each tube contains a single 600-mg section of specially cleaned and dried silica gel with a glass-wool plug and a glass fiber filter at front of the tube before the silica gel, and another glass wool plug at the end of the tube. This method is selected because it has greater sensitivity than OSHA Method 1013. Method 1012 requires the use of an ethanol solution containing a derivatizing reagent to extract and chemically derivative diacetyl in the samples.

Preparation

Before entering the work area all members of the sampling team should be made aware of any requirements for safety equipment such as hair nets, respirators, or safety shoes, and possess all necessary equipment and training, including respirator certification if needed. Procedures and schedules should be coordinated with the analytical laboratory to assure compatibility of procedures and availability of personnel to process samples in a timely manner.

All sampling equipment and supplies should be prepared in advance. Equipment may include battery powered personal sampling pumps capable of operating in the appropriate flow rate range and pressure drop, chargers for those pumps, sample holders of a size compatible with the sampling media, and flexible tubing to connect pumps and sample holders. In this protocol diacetyl and 2, 3-pentanedione vapor samples are collected with two silica gel sorbent tubes in series (SKC Inc., Eighty Four, PA, Catalog no. 226-183). The front tube is connected to the back tube with a piece of tubing to form the sampling train. If the sample holders are not opaque, these sorbent tubes should be wrapped in foil or opaque tape during and after sampling to prevent

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exposure to light. Each sampling tube should be marked with a unique identification number, either before or after sampling. This information is entered in the field data sheet along with the corresponding pump ID, calibrated flow rate, and other information. A useful convention is to mark each of the two tubes of a sample with the same initial identifier, then add an "f" for the front tube and an "r" for the rear tube.

Sampling trains should be assembled and calibrated with sampling media in line, and this sampling media should not be used for any other purpose. Nominal sampling rates for this method are 0.05 Lpm for 180-minute TWA samples and 0.2 Lpm for 15-minute STEL samples. Calibrated flow rate for each pump should be recorded on a field data sheet with an identification code for that pump. A supply of belts, clips, tape, and miscellaneous tools should be available to attach the sampling trains to workers to minimize interference or safety concerns with their jobs.

Collection

To collect samples for the full work shift, the sampling team should be prepared to place sampling trains on the workers as they begin their shifts. Workers and locations to be evaluated should have been previously identified from knowledge of the tasks to be performed and the compounds to be used. A common practice in selecting sampling locations is to choose tasks anticipated to produce the greatest level of exposure to diacetyl or 2, 3-pentanedione and to sample the workers conducting those tasks or collect area samples in those areas. This allows for the greatest likelihood of obtaining samples above the limit of detection for the analytical method, and assumes that if exposure is controlled so that the highest exposures are within allowable limits then all exposures are within those limits.

Immediately before sampling, break off both ends of the flame-sealed tube to provide an opening approximately half the internal diameter of the tube. Attach the tube holder to the worker so that the adsorbent tube is in an approximately vertical position with the inlet in the breathing zone. Position the sampling pump, tube holder, and tubing so they do not impede work performance or safety. As each sampling train is placed and started, the start time should be noted on the field data sheet for that pump, along with the name or other identifier of the person wearing that pump, job title or a description of tasks, and location within the work facility. Sampling site

temperature, relative humidity, barometric pressure, and any other relevant observations should be recorded on field data sheets throughout the duration of sampling. Members of the sampling team should rotate among the sampling locations during the collection of samples. They should occasionally check all sampling devices, observe workers tasks, and note observations on the field data sheet. The use of personal protective equipment and other safety and health controls, ventilation, and all other salient observations should also be noted. Attempt to determine through observation and discussions if workers are engaging in "normal operations."

OSHA states a reliable quantitation limit of 1.3 ppb (4.57 ug/m^3) for diacetyl and 9.3 ppb (38 µg/m^3) for 2, 3-pentanedione with a 180-minute sample duration and a flow rate of 0.05 lpm (or 15 minutes at 0.2 lpm). If the shift being sampled is 8 hours long, three samples approaching 180 minutes would be acceptable to obtain a TWA analyte concentration. These samples should be able to quantify diacetyl and 2, 3-pentanedione at the REL of 5 ppb) TWA or 25 ppb) STEL without exceeding the breakthrough capacity of the sorbent media.

Sampling Surveys

Employers shall conduct exposure monitoring surveys to ensure that worker exposures (measured by full-shift samples) do not exceed the REL, either on a time weighted or short term basis. Because adverse respiratory health effects may occur at the REL, it is desirable to achieve lower concentrations whenever possible. When workers are potentially exposed to airborne flavoring compounds, employers shall conduct exposure monitoring surveys as follows:

- Collect representative personal samples over the entire work shift [NIOSH 1977].
- Perform periodic sampling at least annually and whenever any major process change takes place or whenever another reason exists to suspect that exposure concentrations may have changed.
- Collect all routine personal samples in the breathing zones of the workers.
- If workers are exposed to concentrations above the REL, perform more frequent exposure monitoring as engineering changes are implemented and until at least two consecutive samples indicate that exposures no longer exceed the REL [NIOSH 1977].

- Notify all workers of monitoring results and of any actions taken to reduce their exposures.
- When developing an exposure sampling strategy, consider variations in work and production schedules as well as the inherent variability in most area sampling [NIOSH 1995].

Focused sampling

When sampling to determine whether worker exposures to diacetyl or 2, 3-pentanedione are below the REL, a focused sampling strategy may be more practical than a random sampling approach. A focused sampling strategy targets workers perceived to be exposed to the highest concentrations of a hazardous substance [Leidel and Busch 1994]. This strategy is most efficient for identifying exposures above the REL if maximum-risk workers and time periods are accurately identified. Short tasks involving high concentrations of airborne vapors could result in elevated exposure over full work shifts.

Area sampling

Area sampling may be useful in exposure monitoring to determine sources of airborne diacetyl or 2, 3-pentanedione, and to assess the effectiveness of engineering controls.

Post-collection

After sampling for the appropriate time, remove the sampling train, record stop time, and remove equipment to an uncontaminated area where you can separate the tubes, and seal each tube with plastic end caps. Although tubes were protected from light during sampling, it is also necessary to wrap each tube in aluminum foil or opaque tape making sure that the sample identification number is observable. Samples should be shipped cold (preferable via an overnight carrier) to the accredited analytical laboratory using a "six-pack" cooler and frozen ice packs (Blue Ice) or similar means to refrigerate samples. Submit blank samples as discussed with the laboratory with

each set of samples. Handle the blank samples in the same manner as the other samples except draw no air through them.

Measure the air flow rate through each sampling train (using surrogate sampling media in line, not the actual sample), record this post-sampling flow rate. Determine total sampling time (minutes), mean sampling flow rate, and sample volumes (liters). Place sampling pumps on charge for reuse in required.

Submit the samples to the laboratory for analysis as soon as possible after sampling. As a precaution, store the samples at refrigerator temperature if a delay in shipment is unavoidable. Ship any bulk samples separate from the air samples.

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