UNIVERSITY OF CALIFORNIA

Los Angeles

Development of Air Sampling and Analytical Methods for Acetoin, Diacetyl, and 2,3-Pentanedione

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Environmental Health Sciences

by

Sayaka Takaku-Pugh

2012

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ABSTRACT OF THE DISSERTATION

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Doctor of Philosophy in Environmental Health Sciences

University of California, Los Angeles, 2012

Professor Shane S. Que Hee, Chair

Acetoin, diacetyl, and 2,3-pentanedione are artificial butter flavoring ingredients. Occupational exposures to diacetyl are associated with severe respiratory disease including bronchiolitis obliterans. Dynamic and passive air sampling and analytical methods were developed for simultaneous sampling of these ketones using 10 % (w/w) O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) on Tenax TA (80/100 mesh). PFBHA O-oximes of the ketones were synthesized with above 95 % purity for standards. Ketone vapors of known concentrations and relative humidities (RHs) were generated in Tedlar gas bags for dynamic sampling. A syringe pump delivered ketones in aqueous solution to an air dilution system and an exposure chamber for passive sampling. The dynamic sampling tubes contained 200 mg of coated solid sorbent in a Pyrex tube. The passive samplers were obtained

by compressing 300 mg of coated solid sorbent into a pellet of 30 mm diameter and 0.53 mm thickness. The ketone vapors permeated a silicone membrane and crossed a path length of 3.2 mm to the pellet surface. After sampling, the coated solid sorbent was suspended in water, ultrasonicated, microwaved, extracted with hexane, and centrifuged. An aliquot of the hexane extract was analyzed by capillary gas chromatography-mass spectrometry using selective ion monitoring with the internal standard method. For dynamic sampling, acetoin, diacetyl, and 2,3-pentanedione vapor sampling efficiencies were 90.2 ± 6.9, 92.2 ± 5.9, and 82.5 ± 4.4 % respectively at 5-20 ppb when sampled at 100 mL/min for 8 hours at 25 °C for both 5 and 80 % RHs. Recoveries between 75-125 % were also obtained at 40 ºC at both RHs. For passive sampling, experimental sampling constants for acetoin, diacetyl, and 2,3-pentanedione at 25 ºC for both 5 and 80 % RHs were 59.4 ± 8.5, 55.3 ± 7.6, and 50.0 ± 6.3 mL/min respectively. The experimental sampling constants at 40 ºC were statistically lower at 5 % RH, but had no statistical difference at 80 % RH compared to at 25 ºC. Overall, quantitative, selective, and sensitive dynamic and passive sampling methods were developed around the 2012 ACGIH TLV (10 ppb) for diacetyl in the presence of similar concentrations of acetoin and 2,3-pentanedione.

The dissertation of Sayaka Takaku-Pugh is approved.

William C. Hinds

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Nola J. Kennedy

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University of California, Los Angeles

2012

Dedicated to

My husband, Jeremy L. Pugh

My parents, Etsuo and Sumiko Takaku

My parents-in-law, James and Betty Pugh

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LIST OF ACRONYMS

ACGIH	American Conference of Governmental Industrial Hygienists
ANPRM	Advance Notice of Proposed Rulemaking
APR	air purifying respirator
AUC	area under the curve
Cal/OSHA	California Occupational Safety and Health Administration
CMS	carbon molecular sieve
CV	coefficient of variation
DBP	1,2-dibromopropane
D-diacetyl	di-substituted PFBHA O-oxime of diacetyl
DNPH	2,4-dinitrophenylhydrazine
D-2,3-pentanedione	di-substituted PFBHA O-oxime of 2,3-pentanedione
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FEMA	Flavor and Extract Manufacturers Association
FEV_1	forced expiratory volume in one second
FTIR	Fourier transform infrared
GC	gas chromatography
GC-ECD	gas chromatography using an electron capture detector
GC-FID	gas chromatography using a flame ionization detector
GC-MS	gas chromatography-mass spectrometry
GSD	geometric standard deviation
HPLC	high performance liquid chromatography
ID	inner diameter

IR	infrared
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LLNA	local lymph node assay
LQL	lowest quantifiable limit
M-acetoin	mono-substituted PFBHA O-oxime of acetoin
M-diacetyl	mono-substituted PFBHA O-oxime of diacetyl
M-heptanal	mono-substituted PFBHA O-oxime of n-heptanal
MMAD	mass median aerodynamic diameter
M-2,3-pentanedione	mono-substituted PFBHA O-oxime of 2,3-pentanedione
NIOSH	National Institute for Occupational Safety and Health
OD	outer diameter
OSHA	Occupational Safety and Health Administration
PAPR	powered air purifying respirator
PFBHA	O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride
PID	photoionization detector
PM	particulate matter
ppb	parts-per-billion by volume (unless specified otherwise)
PPE	personal protective equipment
ppm	parts-per-million by volume (unless specified otherwise)
REL	recommended exposure limit
RH	relative humidity
RQL	reliable quantitation limit
SAR	supplied air respirator

SD	standard deviation
SIM	selective ion monitoring
STEL	short-term exposure limit
TIC	total ion current
TLV	threshold limit value
TWA	time-weighted average
UV	ultraviolet
VOC	volatile organic compound

ACKNOWLEDGEMENTS

My sincerest thanks go to my advisor, Dr. Shane S. Que Hee, for all of his time, effort, patience, care, and guidance throughout my research. When research or results went awry, he provided encouragement and support. With his door always open, he was always willing to assist me but ensured that I remained self-sufficient. Without a doubt, he has truly made a profound positive impact on my career.

I would like to extend my gratitude to my wonderful committee members Dr. William C. Hinds, Dr. Thomas R. Belin, and Dr. Nola J. Kennedy for all of their time, advice, and valuable comments. All three are outstanding professors that I truly respect and admire, which is why I was so pleased that they desired to be on my committee.

I appreciate the funding support for fees and tuition from the UCLA Graduate Division, the Fielding School of Public Health, the Department of Environmental Health Sciences, and the personal finances of Dr. Shane S. Que Hee, as well as the research support from the Southern California NIOSH Education and Research Center pilot project grant T420H009412 and NIOSH grant RO1OH009250.

My husband Jeremy L. Pugh already knows how deeply I appreciate his assistance, as I reminded him daily of his positive influence. Without his love, support, and understanding, I would not have completed my doctoral degree. When things became really difficult the last few months of finishing my degree, he took care of everything so I could focus on completing my research. I am grateful for my parents, Etsuo and Sumiko Takaku, and my parents-in-law, James and Betty Pugh. Although they were physically at such a distance, their words of encouragement were able to strengthen my resolve. They continually held the upmost faith in my abilities.

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1 Introduction

Acetoin, diacetyl, and 2,3-pentanedione are used as components of artificial butter flavorings in the flavoring and food industry, including microwave popcorn manufacturing.[1, 2] Occupational exposure to their vapors is associated with various upper and lower respiratory problems, including the severe lung disease bronchiolitis obliterans.[3-12] Thus, monitoring of these ketone vapors is critical to ensure worker health and safety. This research presents methods to measure the concentration of these ketone vapors accurately and precisely under a wide range of environmental conditions, such as at different vapor concentrations, temperatures, and RH.

1.1 Hypothesis

Development of quantitative, selective, and sensitive dynamic and passive air sampling and analytical methods is achievable for simultaneous acetoin, diacetyl, and 2,3-pentanedione vapor sampling using Tenax TA (80/100 mesh) coated by PFBHA.

1.2 Aims

The specific aims included:

1. Development of analytical methods using PFBHA O-oximes as standards for acetoin, diacetyl, and 2,3-pentanedione
2. Development of a dynamic (active) air sampling method using a personal sampling pump
3. Development of a passive (diffusive) air sampling method without a pump using the dynamic method for validation purposes

Pure PFBHA O-oximes for acetoin, diacetyl, and 2,3-pentanedione were required to be synthesized for proper quantitation of the three ketones. To analyze the ketones collected on the solid sorbent in the tubes or passive sampling pellets, reaction efficiencies of the ketones with PFBHA as well as desorption and extraction efficiencies of the PFBHA O-oximes were necessary to determine. Sampling efficiencies at different temperatures, RHs, and sampling durations were necessary to identify for both dynamic and passive sampling. Valid and reliable concentration ranges including sampling tube and pellet capacities and storage periods needed investigation. The design of the passive sampler had to be formulated from diffusion theory, and the experimental sampling constants of the ketone vapors relative to the pellets were required to be obtained.

2 Background

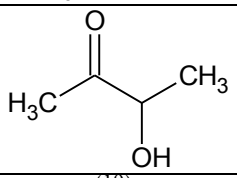
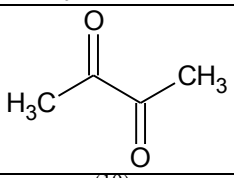
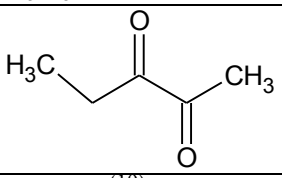
This section provides literature reviews on acetoin, diacetyl, and 2,3-pentanedione. Chemical and physical properties, sources, health effects, exposures, regulations, control and medical surveillance, and current air sampling methods are described. Furthermore, diffusion theory is explained for passive samplers.

2.1 Chemical and Physical Properties of Acetoin, Diacetyl, and 2,3-Pentanedione

Acetoin, diacetyl, and 2,3-pentanedione are all ketones, and Table 2-1 shows their chemical and physical properties. Synonyms for acetoin are acetyl methyl carbinol; acetylmethylcarbinol; 2-butanol-3-one; 2,3-butanolone; 2-butanone, 3-hydroxy-; dimethylketol; 3-hydroxy-2-butanone; 1-hydroxyethyl methyl ketone; *gamma*-hydroxy-*beta*-oxobutane; methanol, acetylmethyl-[13] Synonyms for diacetyl are biacetyl; butadione; 2,3-butadione; butane-2,3-dione; butanedione; 2,3-butanedione; 2,3-biketobutane; dimethyl diketone; dimethylglyoxal; dimethyl glyoxal; glyoxal, dimethyl.[14] Synonyms for 2,3-pentanedione are acetyl propionyl; acetylpropionyl; acetyl propionyl; 2,3-pentadione; pentane-2,3-dione; 2,3-pentanedione[15] Diacetyl and 2,3-pentanedione are α-diketones.

Acetoin can be oxidized to form diacetyl. Acetoin exists as the liquid monomer or the solid dimer.[16-18] Acetoin dimer is converted to monomer by dissolving in water or other solvents or by heating. The proximity of the two carbonyl groups limits reaction at the second ketogroup.

Table 2-1: Chemical and Physical Properties of Acetoin, Diacetyl, and 2,3-Pentanedione

	Acetoin (Monomer)	Diacetyl	2,3-Pentanedione
CAS Number	513-86-0	431-03-8	600-14-6
Molecular Formula	$C_4H_8O_2$	$C_4H_6O_2$	$C_5H_8O_2$
Structural Formula			
Molecular Weight	88.106[19]	86.090[19]	100.117[19]
Color/Form	colorless to pale-yellow liquid[13]	yellow to yellow-green liquid[14]	yellow to yellow-green liquid[15]
Odor	buttery odor[20]; bland, woody, yogurt odor[13]	very strong buttery odor[14]; quinone odor[21]; vapors have a chlorine-like odor[21]; rancid butter odor[21]	sweet odor similar to quinone[15]
Odor Threshold	800 µg/L in water[22]	0.3-15 ppb in air[14]; 0.025 µg/L in air[22]	20 ppb in air[15]; 0.015 µg/L in air[22]
Taste	fatty creamy "tub" butter taste[13]	butter taste[21]	penetrating, buttery taste[15]
Boiling Point	148 °C[19]	88 °C[19]	109.9 °C[19]
Melting Point	15 °C[19]	-1.2 °C[19]	-52 °C[23]
Density	1.0044 g/cm^3 at 20 °C[19]	0.9808 g/cm^3 at 18 °C[19]	0.9565 g/cm^3 at 19 °C[19]
Octanol/Water Partition Coefficient	log K_{ow} = -0.36 (estimated)[20]	log K_{ow} = -1.34[21]	log K_{ow} = -0.85 (estimated)[24]
Water Solubility	miscible [18, 20]; 1 kg/L at 20 °C[25]	200 g/L at 15 °C[21]	66.7 g/L at 15 °C[24]
Vapor Density (air = 1)	3.0 (calculated)	3.0 (calculated)	3.5 (calculated)
Vapor Pressure	2.7 mm Hg at 25 °C (estimated)[20]	56.8 mm Hg at 25 °C[21]; 6.9 kPa at 20 °C[26]	2.67 kPa at 20 °C[27]
Henry's Law Constant	1.0x10^{-5} atm-m^3/mol at 25 °C (estimated)[20]	1.33x10^{-5} atm-m^3/mol at 25 °C[21]	2.62x10^{-7} atm-m^3/mol at 25°C (estimated)[28]
Flashpoint	50.6 °C (closed cup)[17]; 46.7 °C (closed cup)[18]	26.7 °C (closed cup)[17, 18]	19 °C (open cup)[23]
Autoignition Temperature	370 °C[17]	285 °C[17, 18]	265 °C[23]
Lower/Upper Explosive Limit by Volume	1 - 12.2 % [29]	2.4% - 13%[30]	1.8% -10.9 %[31]

2.2 Sources of Acetoin, Diacetyl and 2,3-Pentanedione

Acetoin, diacetyl, and 2,3-pentanedione are found in food and in the environment both naturally and anthropogenically.[13-15] Diacetyl and acetoin are the dominant compounds identified as artificial butter flavorings used in microwave popcorn.[2] 2,3-Pentanedione is also found in flavorings for microwave popcorn when other chemicals were substituted for diacetyl in butter flavorings.[1] Other than in microwave popcorn, these ketones are used as cost-effective artificial flavorings in many food products to impart taste and aroma.

Acetoin is naturally found in fresh apple, butter, cheddar cheese, coffee, cocoa, honey, wheat bread and wine.[13] However, the concentrations of acetoin found in natural substances are low. For example, Australian honey contains 0.5 - 6.9 mg/kg of acetoin and nectarines contain less than 10 mg/kg.[20] In mixture with other products, acetoin is naturally produced via fermentation from the catalytic oxidation of 2,3-butanediol.[13]

Acetoin is also used in alcoholic beverages (3.1 ppm), baked goods such as ready-to-eat and ready-to-bake products, flours, and mixes (380 ppm), breakfast cereals (0.67 ppm), cheese (10 ppm), chewing gum (0.42 ppm), condiments and relishes (2.0 ppm), confection and frosting (21 ppm), fats and oils (50 ppm), frozen dairy (10 ppm), fruit juice (0.03 ppm), gelatins and puddings (81 ppm), grains (200 ppm), gravies (0.029 ppm), hard candy (18 ppm), imitation dairy (50 ppm), meat products (12 ppm), milk products such as flavored milks, milk drinks, dry milks, toppings, snack dips, spreads, and weight control milk beverages (0.012 ppm), nonalcoholic beverages (1.8 ppm), reconstituted vegetables (32 ppm), seasoning and flavors such as all natural and artificial spices and blends (30 ppm), snack foods such as chips and pretzels (36 ppm), soft candy such as candy bars, chocolates, fudge, mints, and other chewy or nougat candies (9.8 ppm), soups (0.05 ppm), and sweet sauce such as chocolate, berry, fruit, maple and corn syrup (98

ppm).[13, 32] The given concentrations in food for acetoin, diacetyl, and 2,3-pentanedione are stated in ppm by mass, and provided as "usual" amount instead of in a range. The food categories above are provided by FEMA and grouped into 34 generic food categories, which are derived from the 43 food categories provided by FDA.

Diacetyl is naturally found in many plants, such as in oils of Finnish pine, angelica, lavender and various flowers.[14] It has been measured in pine, oak, and eucalyptus burned wood emissions at 89, 73, and 73 mg/kg respectively.[21] Diacetyl is also naturally contained in ligonberry, guava, raspberry, strawberry, cabbage, peas, tomato, vinegar, cheeses, yogurt, milk, butter, chicken, beef, mutton, pork, cognac, beer, wines, whiskies, tea, and coffee.[14] Diacetyl is naturally produced due to fermentation of glucose via acetoin.[14]

Diacetyl is used in alcoholic beverages (usual concentration of 6.3 ppm), baked goods (28 ppm), cheese (3.7 ppm), chewing gum (0.69 ppm), fats and oils (6.0 ppm), frozen dairy (11 ppm), gelatins and puddings (13 ppm), gravies (7.2 ppm), hard candy (11 ppm), imitation dairy (11 ppm), meat products (28 ppm), milk products (4.7 ppm), nonalcoholic beverages (10 ppm), snack foods (0.38 ppm), and soft candy (17 ppm).[14]

Diacetyl was identified in surface water at 80 ng/L in Boussy Saint Antoine, France.[21] Diacetyl can be formed as an ozone disinfection by-product in drinking water from its methylglyoxal precursor. [33, 34] Diacetyl is also emitted from motor vehicles[21, 35] and the average on-road concentration from 7 tested vehicles was 0.044 mg/km. It has also been found in cigarette smoke.[36]

2,3-pentanedione is naturally found in the essential oil of Finnish pine, peach, wheaten bread, yogurt, cocoa, coffee, black tea, roasted barley, roasted filbert, roasted peanut, roasted almonds, pecans, soybean, malt, chayote, peas, cooked potato, tomato, butter, boiled egg, fatty

fish, cooked chicken, beef and pork, beer, cognac, rum, whiskies, grape wines, potato chips, passion fruit, mango, beans, macadamia nut, tamarind, sweet potato, pumpkin, sweet corn, shrimp, oyster, okra, clam, mate and soursop.[15]

2,3-pentanedione is also used in alcoholic beverages (usual concentration of 0.62 ppm), baked goods (4.7 ppm), breakfast cereals (12 ppm), chewing gum (0.04 ppm), frozen dairy (4.4 ppm), gelatins and puddings (3.7 ppm), gravies (0.20 ppm), hard candy (40 ppm), meat products (4.4 ppm), nonalcoholic beverages (1.1 ppm), soft candy (4.5 ppm), and sweet sauce (0.30 ppm).[15]

Acetoin is also synthesized from diacetyl by partial reduction with zinc and acid.[13] Diacetyl is also synthesized by converting methyl ethyl ketone into the isonitroso compound, which is decomposed to diacetyl via hydrogen chloride hydrolysis.[14] 2,3-pentanedione is synthesized by oxidation of methyl propyl ketone using excess sodium nitrite and diluted hydrogen chloride with hydroxylamine hydrochloride under a nitrogen atmosphere.[15]

2.3 Health Effects and Case Studies

There is concern about occupational exposures to these ketones in the flavoring and food production industry, because artificial butter flavoring exposures are associated with chronic cough, shortness of breath, asthma, chronic bronchitis, and bronchiolitis obliterans.[3-12] Bronchiolitis obliterans is a relatively rare, severe, incurable, and potentially fatal lung disease that is characterized by airway inflammation and fibrosis at the bronchiolar level.[10-12, 37, 38] The scarring may lead to partial small airway obstruction, or may cause complete obstruction of the lumen.[12] Symptoms of the disease include progressive shortness of breath, a nonproductive

cough, inspiratory grackles, and wheezes. [12, 38] Lung function testing indicates airflow obstruction in FEV_1.[38]

Bronchiolitis obliterans is also known as popcorn worker's lung or popcorn packers' lung[6, 10, 39-42] due to the occurrence of such lung diseases in microwave popcorn plants, but the term more generally refers to a variety of lung diseases. Among cases of popcorn worker's lung, oral corticosteroid and bronchodilator treatments have generally not lessened the obstruction of airways.[5, 43, 44] Thus, a lung transplant is one of the only treatment options for bronchiolitis obliterans.[38]

Other than severe respiratory disease, acetoin, diacetyl, and 2,3-pentanedione exposure can cause eye, nasal, throat, and skin irritation.[12, 26, 27, 45] Nine medical cases of workers in microwave popcorn production were reviewed and all nine had eye irritation, five had nasal irritation, and two workers had skin rashes.[5] Specifically for diacetyl exposure, concentrations higher than 30 ppm caused upper respiratory tract and eye irritation.[46] Patch and maximization testing with diacetyl and acetoin did not produce irritation or sensitization in humans,[41] although there is conflicting toxicological information as mentioned in Section 2.4.4.

Due to the potential health hazards from artificial butter flavorings, NIOSH as well as government and academic researchers have performed field studies to measure air concentrations of flavorings and to investigate the effects of health hazards. Several studies have been performed at microwave popcorn manufacturing plants,[5, 8, 47-55] popcorn popping plants,[7, 43] flavoring manufacturing plants,[3, 56-59] bakery mix production facility,[44, 60] commercial kitchens,[61] and diacetyl manufacturing.[4, 6] From these studies, at least 3 deaths have been reported for workers exposed to diacetyl and other VOCs from butter flavorings at microwave popcorn facilities.[41]

In 1986, NIOSH first reported that catastrophic fixed airway disease had developed in two workers at International Bakers Services, Inc., in South Bend, Indiana.[44] In that factory, the workers weighed and loaded fragrances, flavorings, starch, and flour into one of three mixers. The findings suggested that the workers had bronchiolitis obliterans or emphysema.

In order to find the relationship between lung-function abnormalities and exposure to flavorings, nine medical cases of workers in microwave popcorn production were reviewed, interviews conducted, and serial lung spirometry performed.[5] Out of nine cases, five are on lung transplant waiting lists.

One study analyzed medical and environmental surveys from six microwave popcorn facilities.[48] Investigation revealed that affected workers were exposed to diacetyl concentrations as low as 0.02 ppm. Also, it was identified that airways obstruction and respiratory symptom occurances were higher among flavorings mixers with longer work histories and for workers in packaging areas near tanks containing flavorings. The research suggested that peak exposures may be dangerous even for facilities where low average exposures are maintained through engineering controls such as ventilation. Thus, respirator protection is also necessary, and recommendations to the type of respirator protection are provided in Section 2.6.

In a similar study of the 135 workers (117 completed medical evaluations) at a microwave-popcorn production plant, eight persons had severe bronchiolitis obliterans.[50] In the study, questionnaire responses and spirometry findings from the workers were compared with data from the third National Health and Nutrition Examination Survey and the rates of symptoms and abnormalities were analyzed according to current and cumulative diacetyl exposure. From the study results, workers had 2.6 times the expected rates of chronic cough and shortness of

breath, based on comparisons with national data, and 2 times the expected rates of asthma and chronic bronchitis. Furthermore, workers had 3.3 times the expected rate of airway obstruction, and workers who never smoked had 10.8 times the expected rate. The higher rates of shortness of breath occurred in workers directly involved in the production of microwave popcorn. Importantly, the results showed a strong relation between estimated diacetyl exposure and extent of airway obstruction. Lower mean FEV_1 measurements were associated with greater cumulative exposure to diacetyl.

At one popcorn plant where NIOSH was requested to perform a health hazard evaluation, 10 of 41 workers were suspected of having bronchiolitis obliterans.[51] Cough, chest pain, and shortness of breath were the health concerns raised at the plant and exposures included butter flavorings, coloring agents and salt. Workers participated in a health questionnaire which was compared to national data after controlling for race, age, and smoking status. From the comparisons, employees had chronic cough rates 3 times the national average and non-smokers had about 2 times greater rate of shortness of breath. Furthermore, there was twice the rate of airways obstruction in plant employees overall compared with national rates. Workers who demonstrated airways obstruction did not show signs of reversibility when bronchodilators were used.

Another study was performed on a small family-owned popcorn popping company, where all of the three non-smoking workers developed respiratory disease.[7] Data acquired from interviews, medical records, and high resolution computed tomograms of the chest showed the presence of occupational asthma in all the three workers with possible bronchiolitis obliterans in two of them.

To assess the association between diacetyl exposure and decrease in pulmonary function, spirometry was conducted for 765 full-time employees between 2005 and 2006 at four microwave popcorn production plants.[8] Diacetyl exposures were compared between employees who worked with mixing and nonmixing processes. The mixer employees had a significantly decreased FEV_1 % predicted in non-Asian and Asian males at -6.1 and -11.8% predicted, respectively. On the other hand, nonmixers had no significant impact in decline in FEV_1. There was an eight-fold increased risk for airway obstruction among mixers or workers with cumulative diacetyl exposure higher than 0.8 ppm-year.

In another study, 175 of 196 workers from a chemical production plant that produced diacetyl were interviewed and spirometry was conducted between 1960 and 2003.[6] Acetoin, diacetyl, acetaldehyde, and acetic acid were potential exposures in the plant. Three workers were identified as having bronchiolitis obliterans syndrome based on high-resolution computed tomography of the lungs, two of whom were lifelong nonsmokers.

Furthermore, fixed airway obstruction consistent with bronchiolitis obliterans has also developed in a worker exposed to potato crisp flavoring in a British factory where diacetyl was used.[42] Thus, this case highlights that bronchiolitis obliterans is likely also occurring in countries outside the U.S. and in settings outside the popcorn industry.

In one review of flavoring-related illness, epidemiological factors for clinical bronchiolitis obliterans were provided.[11] From the study, it was derived that age, sex, and duration of employment were not linked with airway obstruction. Also, both smokers and non-smokers had airways obstruction. Furthermore, even at diacetyl exposures of 0.6 ppm, five of six quality control workers had obstruction of airways.

2.4 Exposures

2.4.1 Environmental Exposures

Although acetoin, diacetyl, and 2,3-pentanedione are found in the environment, the chemicals do not accumulate there due to their low ppb - ppm concentrations as mentioned in Section 2.2. Also, volatilization from water surfaces and moist soil is expected to be an important fate process due to their Henry's Law constants as shown in Table 2-1. However, these ketone vapors will be removed rapidly from the atmosphere. Acetoin in air has a short lifetime in air due to reaction with hydroxyl radicals that are photochemically produced.[20] Diacetyl and 2,3-pentanedione also have a short lifetime due to photolysis and reaction with hydroxyl radicals.[21, 62]

2.4.2 Occupational Exposures

Even though these ketone exposures are low in the environment, occupational exposure is higher especially in chemical production and the food flavoring industry. According to Fenaroli's Handbook of Flavor Ingredients published in 2010, the annual consumption of acetoin, diacetyl, and 2,3-pentanedione in the U.S. were 33833 lb,[13] 153500 lb,[14] and 1550 lb[15] respectively.

Diacetyl alone is just one of over 2000 chemicals used by the flavoring industry,[46] but many studies focused on diacetyl to find the concentration in air due to its association with adverse health hazards mentioned in Section 2.3. These studies generally attempted to assess diacetyl concentration in different areas throughout a facility. All of the sampling and analysis methods used in field studies will be explained in more detail in Section 2.7. In some studies mentioned below, NIOSH method 2557, which is a dynamic personal sampling method based on

a CMS, is utilized as shown in Section 2.7.2. This method is substantially affected by RH levels exceeding around 30 % or absolute humidity levels exceeding 6 mg of water per liter air sampled, which causes an underestimation of actual diacetyl concentrations.[63] When the absolute humidities exceeded 8 and 10 mg of water per liter air sampled, the recoveries were below 50 and 30 % respectively. Thus, the concentrations provided in the following example studies may be underestimated, but the data can be compared for different industries.

Four microwave popcorn manufacturing plants were assessed to find diacetyl vapor for several job titles and corresponding tasks, such as carton packer, case packer, crew leader, filler, floater, forklift, stovetop, maintenance, mixer, mixer assistant, palletizer, quality assurance, and sanitation from 2005 to 2007.[64] NIOSH method 2557 was used to find concentrations of diacetyl in personal breathing zones. For the 639 collected samples, job titles were divided into either a mixer who worked in the slurry room mixing vegetable oil, salt, and flavorings or a non-mixer who was not involved in the slurry room processes. Across the four plants (Plant 1, 2, 3, and 4) in the study, the arithmetic mean exposures for mixers were 0.614 ± 0.705, 0.348 ± 0.586, 0.057 ± 0.065, and 0.860 ± 1.048 ppm respectively, compared to 0.031 ± 0.046, 0.074 ± 0.124, 0.027 ± 0.123, and 0.014 ± 0.033 ppm respectively for non-mixers. According to a two-tailed Student t-test at $\alpha = 0.05$, the mean exposures for mixers and non-mixers were significantly different at each plant except for Plant 3. Similarly, the geometric mean exposures (among Plant 1, 2, 3, and 4) for mixers were 0.231 ± 5.52, 0.059 ± 10.4, 0.029 ± 3.81, and 0.230 ± 9.60 ppm respectively, and 0.018 ± 2.74, 0.014 ± 8.97, 0.001 ± 16.4, and 0.003 ± 5.02 ppm respectively for non-mixers. Furthermore, the concentration range for mixers was 0.004-3.900 ppm, and 0.004-1.000 ppm for non-mixers. Importantly, after engineering controls were implemented, diacetyl exposure in one slurry room decreased from 0.614 to 0.061 ppm. The data correlated increased

airway obstruction with high-exposure job duties such as mixers and mixer assistants, with a detailed respiratory morbidity study detailed by Lockey et al[8] as mentioned in Section 2.3.

In another study, diacetyl air concentrations at six microwave popcorn plants were obtained in mixing and packaging areas.[48] NIOSH method 2557 was used to find concentrations of diacetyl in several areas as indicators of exposure to butter flavoring chemicals. Across the six plants (Plant A, B, C, D, E, and F) in the study, the arithmetic mean concentrations of diacetyl were 37.8, 0.6, 0.4, 0.2, 0.6, and 1.2 ppm respectively in mixing areas, and 1.9, 0.7, 0.03, 0.004, 0.3, and 0.02 ppm respectively in packaging areas. At most plants, personal exposure measurements of diacetyl were also obtained. The arithmetic mean diacetyl personal exposures at Plant B, C, D, E, and F were 0.6, 0.03, 0.02, 0.4, and 1.0 ppm respectively in mixing areas, and 0.5, 0.02, 0.002, 0.6, and 0.02 ppm respectively in packaging areas. Results indicated higher prevalence of airways obstruction and respiratory symptoms in mixers as mentioned in Section 2.3.

In a microwave popcorn packaging plant, ketone compounds including diacetyl and acetoin in air were analyzed using NIOSH Method 2557 and 2558 respectively.[2] Out of a total of 53 diacetyl area samples, diacetyl vapor concentrations ranged from below detectable limits (<0.01 ppm) to 98 ppm with an arithmetic mean of 8.1 ± 18.5 ppm and a geometric mean of 0.71 ± 14.4 ppm.[2] Out of a total of 53 acetoin area samples, acetoin vapor concentrations ranged from below detection limits (<0.02 ppm) to 12 ppm with an arithmetic mean of 0.92 ± 2.33 ppm and a geometric mean of 0.10 ± 7.93 ppm. The arithmetic mean diacetyl concentration of 37.8 ± 27.6 ppm was the highest in the mixing room compared to the other area samples. Similarly, the arithmetic mean acetoin concentration of 3.9 ± 4.3 ppm was the highest in the mixing room. In

this plant, eight former workers were reported to have bronchiolitis obliterans with four workers being placed on lung transplant waiting lists.

This study also determined concentration of 2-nonanone, methyl ethyl ketone, acetaldehyde, and acetic acid, as well as total organic vapors (VOCs).[2] Qualitative sampling for VOCs using a portable gas chromatograph with photoionization detector identified over 100 different compounds within the microwave processing area. The predominant vapors were diacetyl, methyl ethyl ketone, acetoin, 2-nonanone, and acetic acid. The total VOC concentration was also highest in the mixing operation room.

In the same facility, total dust concentrations in 55 area samples (full-shift, TWA) ranged from below detection limit (< 0.007 mg/m^3) to 1.0 mg/m^3, with an arithmetic mean of 0.24 ± 0.19 mg/m^3 and a geometric mean of 0.18 ± 2.40 mg/m^3.[2] Respirable dust concentrations from 140 samples (personal and area samples) ranged from below detection limit (< 0.007 mg/m^3) to 0.76 mg/m^3, with a arithmetic mean 0.13 ± 0.11 mg/m^3 and a geometric mean of 0.10 ± 2.23 mg/m^3. Particle size distributions in the microwave mixing room were unimodal, and majority of the particles were less than 10 µm with MMADs of 2.3-5 µm. However, no study was performed to determine whether the particles contained diacetyl or acetoin. The potential for respiratory health effects from such particles containing diacetyl needs further study, especially those particle sizes which can deposited in the bronchioles.

Another study was performed on 16 small- to medium-sized flavor facilities to monitor potential diacetyl exposures.[46] In this study, both personal and area air samples were obtained using NIOSH method 2557. Samples were collected in liquid and powder compounding areas, the research laboratory, and the quality control room at the facilities. A total of 181 diacetyl samples were obtained in the 16 plants, including 105 personal samples and 76 area samples.

Among 105 personal samples collected, the range of diacetyl concentrations was from < 0.01 ppm to 60 ppm where 46 samples were less than 0.01 ppm. The arithmetic mean of the personal samples was 2.48 ppm and the median was 0.14 ppm. Among 76 area sample collected, the range of diacetyl concentration was from < 0.01 ppm to 11 ppm where 46 samples were less than 0.01 ppm. The arithmetic mean of the area samples was 0.91 ppm and the median was 0.07 ppm. The mean diacetyl concentration for all processes was 1.80 ppm and the median was 0.10 ppm. In 6 flavor facilities, real-time samples were collected using a photoacoustic IR spectrometer. The maximum diacetyl concentration obtained from real-time samples was 525 ppm during powder operations.

A chemical production plant that produced diacetyl between 1960 and 2003 was studied to assess exposures from diacetyl.[6] In this plant, diacetyl was produced through oxidation of 2,3-butylene glycol into acetoin, then acetoin was partly oxidized into diacetyl. Acetaldehyde and acetic acid were side products in this production process. Process operators were only exposed to diacetyl at the end of the production process. There was no exposure to heated diacetyl. Limited routine exposure monitoring (26 ambient samples and 4 personal task based samples) was performed using cartridges containing silica gel coated with DNPH. The samples were then analyzed using GC. From the area sampling, the diacetyl air concentration range was from 1.8 to 351 mg/m^3 (0.51-100 ppm). In 2001, the company utilized exposure control, and the diacetyl geometric mean concentration decreased from 10.0 to 5.8 mg/m^3 (from 2.84 to 1.6 ppm). From the personal task-based sampling, the diacetyl air concentration range was from 3 to 396 mg/m^3 (0.85-112 ppm).

From most studies, less information is available regarding peak exposures. However, headspace sampling from in-tank at one studied microwave popcorn plant helps provide insight

into peak exposures. From the study, mixers may have had brief diacetyl exposures as high as 1230 ppm or acetoin exposures of 972 ppm when opening certain tank lids containing heated flavoring.[55]

Due to health concerns posed by diacetyl, flavor manufacturers have started to substitute chemically similar diketones for diacetyl in butter flavorings.[1] Thus, several studies were performed to find what kinds of substitutes were used in flavorings. For example, bakeries that utilize artificial buttermilk flavorings have been investigated to determine diacetyl and diacetyl substitute concentrations in air.[65] In 2008, NIOSH observed a bakery facility that used multiple buttermilk flavorings including some with diacetyl substitutes with a focus on measuring ketones through several methods. The headspaces of six bulk flavorings were evaluated for VOCs. Area and personal samples were obtained to find ketones during batch preparation. NIOSH 2549, Modified OSHA PV2118, OSHA 1013, NIOSH Draft Procedure SMP2, and evacuated canisters were used to analyze air samples as mentioned in Section 2.7. Out of the five buttermilk flavorings, diacetyl was present in four, acetoin in two, 2,3-pentanedione in four, 2,3-hexanedione in one, and 2,3-heptanedione in three. In the substitute flavoring headspace, 2,3-pentanedione was the predominant ketone. 2,3-pentanedione air concentrations were 78 and 91 ppb for area and personal samples respectively. Diacetyl and acetoin concentrations were less than the minimum detectable concentrations, 47 ppb and 93 ppb respectively, for all personal and area samples.

Similarly, NIOSH was asked to observe chemicals in eight butter flavorings at one microwave popcorn plant.[1] The plant had been informed by their flavoring supplier that diacetyl substitutes were being utilized, but the popcorn plant was not informed of the chemical composition of the substitute. Liquid butter flavoring samples were collected at the plant and

analyzed with GC-MS and a semi-quantitative headspace analysis was performed on the samples using a thermal desorption tube method. From the GC-MS analysis of the 8 butter flavorings, acetoin, 2,3-pentanedione, and 2,3-hexanedione were found in 5, 4, and 1 flavorings respectively with diacetyl and 2,3-heptanedione being undetected. With the exception of one sample maintaining a 2% level of acetoin by weight, chemical concentrations of α-diketones were 0.5% or less by weight. Through analysis via the semi-quantitative method, acetoin and diacetyl were detected in all 8 samples with 2,3-pentanedione, 2,3-hexanedione, and 2,3-heptanedione in 5, 1, and 1 butter flavorings respectively.

2.4.3 Consumer Exposures

There is limited exposure assessment in relation to consumers of food containing artificial butter flavorings. However, Dr. Cecile Rose at the National Jewish Medical and Research Center, wrote letters to the FDA, Centers for Disease Control and Prevention, EPA, and OSHA about a patient who developed significant lung disease while receiving daily inhalation exposure as a heavy consumer of butter flavored microwave popcorn.[66]

The patient daily consumed several bags of extra butter flavored microwave popcorn for several years. The patient experienced a worsening cough, shortness of breath, and a progressive decline in FEV_1. Lung biopsy showed signs of bronchiolitis obliterans including hyperinflation, absence of small airways, and obliterated bronchioles. In order to find the diacetyl concentration in the patient's home, airborne levels were measured during microwave popcorn preparation. Although no specific vapor exposure concentrations were mentioned in the letter, the diacetyl concentration identified was similar to the microwave oven exhaust area of a microwave popcorn manufacturing plant.

Thus, exposures of artificial butter flavorings may not be just limited to workplace settings. However, acetoin, diacetyl, and 2,3-pentanedione exposures to production workers and consumers are different in terms of concentration and duration. For example, consumers are exposed to the ketones during cooking and eating their food. On the other hand, production workers could be exposed to the chemicals during entire work shifts.

Since consumers may be exposed to artificial butter flavorings in their home, it is important to assess the impact on indoor air quality from microwaving popcorn. In order to find the actual concentration of VOCs and PM released from the process of cooking microwave popcorn, one study analyzed 17 types of microwave popcorn.[67] The research identified emissions during popping opening bags (Phase 1) and post-pop opening at intervals ranging from 0-40 min.

Air was sampled using the EPA Method TO-17[68] to identify and quantify VOCs in the Tenax TA tube air samples.[67] Sep-Pak DNPH silica gel cartridges were used along with Tenax TA, since Tenax TA was not suitable for all compounds containing carbonyl groups, and analysis was performed via EPA method IP-6A.[69] Research observations found average emissions of diacetyl to be 778.9 ± 135 μg emitted/bag of popcorn. Also, numerous VOCs were detected from the study, with butyric acid being in the highest concentration followed closely by 0.5-1.0 ng/mL (0.14-0.28 ppm) of diacetyl and acetoin. However, the study did not detect 2,3-pentanedione. In addition to butter flavorings, bag components such as p-xylene and perfluorinated alcohol 8:2 telomer were also found.

An aerodynamic particle sizer and a scanning mobility particle sizer were also utilized to find emitted aerosol sizes.[67] For PM, the average concentration emitted in the 515 liter chamber during popping and opening was 1900 $\mu g/m^3$, with a range from 0.76 $\mu g/m^3$ to 3100 $\mu g/m^3$. The

PM emissions were 99% within the respirable range (< 4 μm). Results showed a bimodal size distribution with a MMAD of 0.23 and GSD of 1.7 for fine particles; whereas, coarse particles had a MMAD of 1.72 and GSD of 1.7.

2.4.4 Toxicology

Because artificial butter flavoring is associated with respiratory diseases, it is important to understand the toxicological effects at different doses and durations. Currently, the toxicity mechanisms remain controversial. There are several animal studies assessing butter flavoring inhalation exposure effects using mice[70, 71] and rats.[72-75]

In order to find the respiratory toxicity of diacetyl, male C57Bl/6 mice were exposed to diacetyl to simulate workplace conditions at microwave popcorn packaging plants.[70] The mice were subjected to different routes, diacetyl doses, and durations. Respiratory tract effects from diacetyl inhalation or direct oropharyngeal aspiration were evaluated by histopathology and bronchoalveolar lavage fluid analysis. Subacute diacetyl exposure at 200 or 400 ppm for 5 days caused deaths, necrotizing rhinitis, necrotizing laryngitis and bronchitis.[70] Although less nasal and laryngeal toxicity occurred by reducing the diacetyl exposure to 1 h/day (100, 200, 400 ppm) for 4 weeks, this exposure reduction caused peribronchial and peribronchiolar lymphocytic inflammation. Via the intermittent high-dose inhalation regime (1200 ppm), similar results were observed. Moderate nasal injury, peribronchial lymphocytic inflammation, epithelial atrophy, denudation, and regeneration occurred from subchronic exposures at 100 ppm. When bypassing the nose via oropharyngeal aspiration, diacetyl doses of 400 mg/kg caused foci of fibrohistiocytic proliferation with little inflammation at the alveolar duct and terminal bronchiole junction.

Overall findings from the study indicated that diacetyl caused injury similar to bronchiolitis obliterans in humans.

Although none of the mice were diagnosed with bronchiolitis obliterans, the results suggested that workplace exposure to diacetyl contributes to the development of bronchiolitis obliterans in humans because lymphocytic bronchiolitis is a potential precursor of bronchiolitis obliterans.[70, 76] Because humans and mice have anatomical differences, it was inferred that the nasal cavity of mice was less susceptible to the vapors than that of humans. Furthermore, the reaction of diacetyl vapors in the upper airways of mice might have halted penetration of toxic concentrations to bronchioles or other deep lung tissue where the obstructions in humans are typically found.

Another study also focused on acute airway effects of diacetyl in inbred BALB/cJ male mice.[71] The study evaluated the acute warning properties of sensory irritation (eye, nose, and throat irritation) that could be useful to prevent workers being exposed to high concentrations. Also, the possibility of limitation of airflow and pulmonary irritation was investigated at higher exposure concentrations. From the findings, irritation was induced over all parts of the respiratory tract depending on the diacetyl concentration over a 2 hour period and sensory irritation in humans was estimated to occur above 20 ppm. However, diacetyl levels of 2 ppm have been shown to cause bronchiolitis obliterans in humans. Therefore, no acute warning signal is expected in humans at diacetyl levels that have caused bronchiolitis obliterans. Sensory irritation effects faded quickly but occurred rapidly upon exposure. Upon repeat exposures, diacetyl exposures at a high concentration decreased the sensory irritation warning signal.

Male Sprague-Dawley rats were exposed to heated butter flavoring vapors for 6 hours via inhalation and were necropsied 1 day following exposure.[73] GC-MS was utilized to determine

exposure constituents including diacetyl, acetic acid, acetoin, butyric acid, acetoin dimers, 2-nonanone, and alkyl lactones. At 285 to 371 ppm of diacetyl, butter flavoring as a mixture caused multifocal, necrotizing bronchitis within the mainstem bronchus of the lung with alveoli remaining unaffected. Necrosuppurative rhinitis occurred due to exposure to butter flavoring vapors containing 203–371 ppm diacetyl. Overall, the study concluded that butter flavoring vapor concentrations were linked with nasal and pulmonary airway epithelial injury in rats.

In another experiment, respiratory toxicologic pathology of inhaled diacetyl was studied in Sprague-Dawley rats.[74] In one group of rats, exposure up to 365 ppm (TWA) was administered either for six continuous hours or four brief, intense exposures over six hours. Rats were necropsied after 18 to 20 hours of exposure. Epithelial necrosis and suppurative to fibrinosuppurative inflammation were observed in the nose, larynx, trachea, and bronchi due to diacetyl inhalation. At diacetyl concentrations of 224 ppm for 6 continuous hours of exposure, the trachea and larynx were affected in 5 out of 6 rats. At diacetyl concentrations of 356 ppm for 6 continuous hours of exposure, the trachea and larynx were affected in 6 out of 6 rats. Furthermore, the research identified that pulsed and continuous exposure patterns both caused epithelial injury and diacetyl sensitivity was greatest for the nose. The overall results concluded that inhaled diacetyl is a respiratory hazard.

In another study, male Sprague-Dawley rats were delivered diacetyl by intratracheal instillation to determine if the exposure would cause bronchiolitis obliterans.[77] Diacetyl was dissolved in sterile distilled water (188 mg/mL), and the rats were either treated with a single 125 mg/kg dose of diacetyl or sterile water as a control. Airway specific injury occurred after instillation of diacetyl which was then followed by rapid epithelial regeneration, and extensive fibrosis of intraluminal airways which is characteristic of bronchiolitis obliterans. Upon

development of bronchiolitis obliterans, the rats showed increased airway resistance and lung fluid neutrophilia similar to how the disease occurs in humans.

Another study utilized male Sprague-Dawley rats to model the diacetyl and butyric acid uptake in humans[75] The developed hybrid computational fluid dynamic-physiologically based pharmacokinetic model simulates the uptake of diacetyl and butyric acid vapors, alone and in combination as observed in the upper respiratory tract. Applying the developed model to humans suggested that inhaled diacetyl may penetrate deeper into the pulmonary airways to a greater degree than found in the rats. Therefore, human intrapulmonary airway injury may be predicted by extrapulmonary airway injury in rats. Similar modeling studies have been undertaken utilizing F344 rats to model respiratory tract uptakes of diacetyl in humans.[72]

There are several toxicology studies on diacetyl exposure, but few for 2,3-pentanedione. This is troubling as 2,3-pentanedione has rapidly become a substitute for diacetyl in artificial butter flavoring. Compared to diacetyl, 2,3-pentanedione shared the same functional alpha-diketone group and might share a similar mechanism of toxicity. Thus, substitutes should not be assumed as safe.

One study on 2,3-pentanedione respiratory toxicity utilized male Sprague-Dawley rats where the rats inhaled 0, 118, 241, 318 or 354 ppm 2,3-pentanedione for 6 hr.[78] The nose, trachea, and lung of the rats were analyzed by histopathology. Nasal epithelium was most affected and degeneration, apoptosis, necrosis and neutrophilic inflammation were the observed airway epithelial changes which increased in severity as exposure concentration increased. Furthermore, injury extended deeper into the respiratory tract upon increasing concentration. At 354 ppm, necrosuppurative tracheitis was identified in all rats and there was delayed onset of toxicity upon physical examination. In summary, the research demonstrated a similarity in

airway epithelium injuries in rats caused by inhaled 2,3-pentanedione as compared to diacetyl. Since the trachea of all rats were affected at 356 ppm of diacetyl[74] and 354 ppm of 2,3-pentanedione, threshold effect of trachea due to the two chemicals were similar.

Acute animal toxicity parameters, such as LD_{50} and LC_{50} for acetoin, diacetyl, and 2,3-pentanedione are shown in Table 2-2.

Table 2-2: Animal Toxicity Values of Acetoin, Diacetyl, and 2,3-Pentanedione

Animal	Test Type	Route	Reported Dose
Acetoin			
Rat	LD_{50}	oral	> 5 g/kg[25]
Rabbit	LD_{50}	skin	> 5 g/kg[25]
Rat	LD_{Lo}	subcutaneous	14 g/kg[25]
Diacetyl			
Guinea pig	LD_{50}	oral	990 mg/kg[79]
Mouse	LD_{50}	oral	250 mg/kg[80]
Rat	LD_{50}	oral	1580 mg/kg[79]
Rat	LD_{50}	oral	3.0 g/kg < LD_{50} < 3.4 g/kg[81]
Mouse	LD_{50}	intraperitoneal	249 mg/kg[80]
Rat	LD_{50}	intraperitoneal	0.40 g/kg < LD_{50} < 0.65 g/kg[81]
Rabbit	LD_{50}	skin	> 5 g/kg[79]
Rat	LC_{50}	inhalation	639 ppm < LC_{50} <1477 ppm for 4 hours[82]
2,3-Pentanedione			
Rat	LD_{50}	oral	3 g/kg[24]
Rabbit	LD_{50}	skin	> 2500 mg/kg[24]

Acetoin showed no mutagenic effect using Ames test in *Salmonella typhimurium* strains TA98, 100 and 102 at doses 0.005-500 µmol/plate both with and without metabolic activation.[83] Acetoin also showed no mutagenic effect using Ames test in *Salmonella typhimurium* strains TA98, 100, 1535, 1537, and 1538 as well as in *Escherichia coli* strain WP2 UVRA at doses 1-5000 µg/plate without metabolic activation.[83] When acetoin (12.0 or 60.0 g/kg) was given intraperitoneal 3 times per week for 6-7 weeks, no carcinogenic activity was observed in mice.[84]

2,3-Pentanedione showed no mutagenic effect using Ames test in *Salmonella typhimurium* strains TA98, 100 and 102 at doses from 0.009 to 900 μmol/plate both with and without metabolic activation.[85]

Diacetyl showed no mutagenic effect using Ames test in *Salmonella typhimurium* strains TA98 and 100 at doses 0.002-200 μmol/plate both with and without metabolic activation.[86] Diacetyl also showed no mutagenic effect using Ames test in *Salmonella typhimurium* strains TA98, 100, 1535, 1537 and 1538 as well as in *Escherichia coli* strain WP2 UVRA at doses 1-5000 μg/plate without metabolic activation.[86] However, diacetyl showed mutagenic effect in *Salmonella typhimurium* strain TA102 at doses 0.002-200 μmol/plate both with and without metabolic activation, in strain TA100 at doses 0-12 μmol/plate without metabolic activation, and in strain TA104 at doses 0-1.75 μmol/plate without metabolic activation.[86]

Diacetyl induced unscheduled DNA synthesis in the organs of various laboratory animals as well as sister chromatid exchanges in Chinese hamster ovary AUXB1 cells.[21] The carcinogenicity of diacetyl and acetoin has been investigated. When diacetyl (1.70 or 8.40 mg/kg) was given intraperitoneal once weekly for 24 weeks, no lung tumors were induced in mice.[21]

One study evaluated diacetyl for mutagenicity through the mammalian cell gene mutation assay in L5178Y mouse lymphoma cells.[87] In the presence of human liver S9 for activation, diacetyl provoked a high level of mutagenic response in the L5178Y mouse lymphoma mutation assay. The study indicated that damage to multiple loci on chromosome 11 as well as functional loss of the thymidine kinase locus occurred due to diacetyl.

Oxidative stress is a possible mechanism for diacetyl to cause lung damage.[41] Diacetyl and its iminium derivatives were observed in a favorable range for catalytic electron transfer in vivo. Thus, as a result of redox cycling, oxidative stress can occur via reactive oxygen species.

Diacetyl was also predicted as a sensitizer using quantitative structure-activity relationship modeling and confirmed via LLNA.[88] Furthermore, diacetyl is also a suggested T-cell mediated chemical sensitizer based on the results of the LLNA, phenotypic analysis, and total IgE dose-response studies.

2.5 Regulations

The FDA regulates diacetyl in the United States and considers it a direct food substance affirmed as generally recognized as safe.[89] Similarly, the FDA regulates acetoin as synthetic flavoring substances and adjuvants for human consumption.[90] Acetoin and diacetyl are also regulated by the FDA as synthetic flavoring substances and adjuvants for animal drugs, feeds, and related products.[91] However, a FEMA review indicated that acetoin and diacetyl along with 32 other flavoring chemicals could present a respiratory hazard if unsafely handled.[92]

There is currently no OSHA permissible exposure limit for acetoin, diacetyl, and 2,3-pentanedione, but OSHA issued a Hazard Communication Guidance for Diacetyl and Food Flavorings Containing Diacetyl on September 24th, 2007.[93] The guidance is not a legal standard or regulation, but it provides a guideline for managing artificial butter flavoring exposure in the workplace. OSHA releFased an ANPRM on Occupational Exposure to Diacetyl and Food Flavorings Containing Diacetyl on January 21st, 2009.[94] The proposal does not provide any regulations or standards, but seeks to have the public provide feedback on diacetyl exposure concerns. However, OSHA withdrew its ANPRM on March 17th, 2009 in order to focus on

producing standards more quickly.[95] OSHA also provided a National Emphasis Program on microwave popcorn processing plants that became effective January 18th, 2011, which outlines a need to reduce or eliminate chemical exposure from butter flavorings.[37]

The NIOSH REL, STEL, and immediately dangerous to life or health for acetoin, diacetyl, and 2,3-pentanedione have not officially implemented yet. However a criteria document draft, "Criteria for a Recommended Standard: Occupational Exposure to Diacetyl and 2,3-pentanedione, " was released on August 12th, 2011 for external review.[96] According to the draft, the NIOSH REL for diacetyl may be 5 ppb TWA during a 40-hour work week and the NIOSH STEL may be 25 ppb for a 15-minute time period. The NIOSH REL for 2,3-pentanedione may be 9.3 ppb TWA during a 40-hour work week and the NIOSH STEL may be 31 ppb for a 15-minute time period. Both guidelines are based on the best available sampling and analysis technology rather than health effects, a procedure commonly adapted for carcinogens and sensitizers. Although the REL and STEL for 2,3-pentanedione are higher than that for diacetyl, it is due to limitations in the air sampling and analytical method and does not reflect relative potential health hazards. For diacetyl, NIOSH recommends an action level of 2.6 ppb, but 2,3-pentanedione does not have an action level because the REL is established at the reliable quantification limit.

Neither the American Industrial Hygiene Association workplace environmental exposure level nor the ACGIH TLV for acetoin and 2,3-pentanedione have been published yet. However, the intended ACGIH TLV for diacetyl was proposed in 2011.[97] The proposed TLV-TWA of 0.01ppm (0.04 mg/m^3) and TLV-STEL of 0.02 ppm (0.07 mg/m^3) were recommended for occupational diacetyl exposure. Diacetyl was listed tentatively as not classifiable as a human

carcinogen. Then, in 2012, the TLV-TWA and TLV-STEL for diacetyl was adopted as proposed in 2011.[98]

The California Code of Regulations, Title 8, §5197 (Occupational Exposure to Food Flavorings Containing Diacetyl), became operational on December 2nd, 2010 and made California the first state to have such regulation.[99] This regulation by Cal/OSHA applies to employers that use or manufacture products containing diacetyl at a concentration of 1% or more by weight. Furthermore, employers are required to report occurrences of fixed obstructive lung disease within 24 hours of becoming aware of the diagnosis. Employers must formulate a written control program and effectively implement engineering controls to reduce employee exposure to diacetyl concentrations in the air. The regulation also mandates different types of respirator use at different diacetyl concentration levels. Employers are also required to provide medical surveillance of workers such as pulmonary function tests, health questionnaires, and medical removal for employees. Title 8, §5197 also mandates specific respirator usage at different diacetyl concentration ranges as shown in Section 2.6.

2.6 Control and Medical Surveillance

Protecting workers from exposures to chemical flavorings is critical as there are currently multiple studies that link flavorings to severe lung disease and health hazards such as bronchiolitis obliterans. Several government agencies and researchers have proposed methods of limiting exposures to butter flavorings such as utilizing chemical substitutes, administrative and engineering controls, worker education programs, personal protective equipment (PPE), exposure monitoring, medical surveillance, and mixtures of all these strategies. For example, the NIOSH alert on "Preventing Lung Disease in Workers Who Use or Make Flavorings," and

OSHA's "Occupational Exposure to Flavoring Substances: Health Effects and Hazard Control" documents outline controls relating to flavoring exposures. [100, 101] In general, the best controls will address both peak and cumulative exposure.

The typical first step for controlling exposure is chemical substitution to reduce health risk. However, substitution poses the same problem in the butter flavorings industry as many of the VOCs within the flavorings have not been assessed as to their safety or health impacts. Importantly, many of the potential flavoring substitutes for diacetyl may also carry the same mechanisms for toxicity since 2,3-pentanedione and other α-diketone compounds have similar chemical structure.[65] Therefore, engineering and administrative controls would likely be the best course for limiting exposure.

As detailed in the NIOSH alert, engineering controls for butter flavorings include ensuring proper ventilation, closed environments for hazardous processes, isolation of high exposure areas, and temperature controls.[100] Temperature controls are useful to limit flavoring exposures because lower temperatures keep chemicals from volatilizing. Proper ventilation such as simple exhaust hoods, ventilated booths, and measuring hood airflows are critical as they can dramatically lower flavoring chemical exposure.[102] Administrative controls should be coupled with ventilation engineering controls to ensure that flavoring containers are tightly sealed and air exposures are reduced.

Furthermore, access to areas where flavorings are handled via open processes should be administratively restricted to allow only essential workers with PPE such as eye, skin, and respiratory protection. For example, Cal/OSHA regulations mandate specific respirator usage at different diacetyl concentration ranges as shown Table 2-3.[99]

Table 2-3: Respiratory Protection Selection

Maximum Diacetyl Concentration	Type of Respirator
Less than or equal to 0.2 ppm, no exposure to diacetyl-containing powders	Half mask respirator
Less than or equal to 0.5 ppm	Any PAPR, SAR, or full facepiece APR
Less than or equal to 1.0 ppm	Full facepiece APR, any tight-fitting PAPR, or SAR
Less than or equal to 20 ppm	Tight fitting full facepiece PAPR or SAR in continuous flow or pressure demand mode, or PAPR or SAR with helmet or hood in continuous flow mode which have been found to provide a protection factor of 1000
Above 20 ppm	Self-contained breathing apparatus in pressure demand mode

To find the air concentration of butter flavorings, area and personal exposure monitoring are necessary. The current sampling and analysis methods available are discussed in Section 2.7. Exposure monitoring along with medical surveillance assess the effectiveness of controls. Regularly scheduled medical surveillance includes spirometry testing and health surveys as worker health is the most important assessment of effective controls. NIOSH provides links to detailed information on spirometry training and monitoring programs.[103]

Furthermore, biomarkers can identify possible health effects from flavoring vapor exposures. Exhaled nitric oxide has been studied as a possible biomarker associated with exposure levels, respiratory symptoms, or airways obstruction.[104] In this study, 135 workers were provided questionnaires, spirometry tests, and exhaled nitric oxide measurements. Although exhaled nitric oxide was significantly lower in workers with high flavoring exposures, it was not identified as a useful biomarker. Induced sputum parameters have also been assessed as possible biomarkers.[105] In another study, a questionnaire, spirometry, and sputum induction was conducted to 59 workers with high exposures and 22 patients with low exposures to

flavoring vapors. Popcorn production workers also had higher measurements of sputum interleukin-8 and eosinophil cationic protein compared to low exposure workers.[105]

2.7 Current Air Sampling Methods

To understand the association of health effects and concentrations, it is critical to know the concentration of chemicals in the air using accurate and precise air sampling methods. Many work places may contain several artificial butter flavorings other than acetoin, diacetyl, and 2,3-pentanedione. Thus, methods for each specific compound and for ketones are reviewed in this section.

Acetoin, diacetyl, and 2,3-pentanedione can be sampled and analyzed using environmental air samples or personal breathing zone samples. There are direct-reading instruments that can provide real-time monitoring of flavoring concentrations. Alternatively, there are integrated samplers that remove contaminants in a known volume of air over a period of time via absorption or adsorption.

Integrated sampling includes dynamic (active) or passive (diffusive) sampling. In dynamic sampling, a known amount of contaminated air is drawn into a sorbent tube, treated filter, or impinger using a pump with calibrated flow rate and known sampling time. On the other hand, passive sampling does not require active movement of air via a pump due to the physical process of diffusion. However, passive sampling is generally less sensitive than dynamic sampling.

There are different types of sampling media. For example, various solid sorbents, such as, activated charcoal, silica gel, Tenax, Chromosorb, and Amberlite are used depending on the required sampling for both dynamic and passive samplers.[106] Similarly to absorb chemicals in

air, various liquid media are used in an impinger depending on the target chemical by passing the air through the impinger solution. In both solid sorbents and liquid media, physisorption and chemisorption are two processes that can collect chemicals from air. Physisorption involves absorption (solubilization) or adsorption of chemicals, resulting in determination of the actual chemical vapor concentration upon analysis. On the other hand, chemisorption involves chemical reaction between the contaminants in air and the chemical coating the sampling media, resulting in analysis of derivatives of collected chemicals. Both methods have their advantages and disadvantages as mentioned in Sections 2.7.2 and 2.7.3.

2.7.1 Direct Reading Methods

There are real-time or direct reading instruments that can monitor VOCs in air, including acetoin, diacetyl and 2,3-pentanedione. They can provide continuous exposure monitoring and can highlight concentration variations over any given time frame, although some instruments cannot detect low concentrations such as at ppb levels.

At one plant, diacetyl concentration was measured in a worker's breathing zone using a FTIR Gas Analyzer.[48] FTIR allowed real-time measurement of diacetyl concentrations when the worker handled open butter flavorings container. Another study also used a real-time FTIR to detect diacetyl in workspace air.[7] FTIR can detect multiple compounds simultaneously at the ppb level.[107] FTIR can identify unknown compounds in mixtures by their absorption versus transmitted radiation pattern at the "fingerprint" region, since IR radiation is absorbed by chemical functional groups at characteristic frequencies.

Because acetoin, diacetyl, and 2,3-pentanedione have at least one carbonyl group, all three compounds have a strong peak on their IR spectrogram around a wavenumber of 1730 cm^{-1}

(wavelength of 5.8 μm).[108-110] Other main peaks for acetoin include 1130, 1380, 3000, and 3500 cm^{-1}.[108] Other main peaks for diacetyl include 900, 920, 1100, 1340, and 1400 cm^{-1}.[109] Other main peaks for 2,3-pentanedione include 900, 1100, 1350, and 3000 cm^{-1}.[110] The intensity of the peak can be used to quantify the concentration of a target compound. Also, lower limits of detection and greater sensitivity is achieved as the length of the path that the IR light travels increases before it reaches the detector. In a portable IR photometer designed for gas analysis, a series of reflecting mirrors permits the path length of the cell to 20 m in increments of 1.5 m.[111]

Another direct reading instrument used in field is a photoacoustic IR spectrometer. Photoacoustic spectroscopy utilizes IR radiation or sound and UV radiation to quantify air contaminants.[107] The molecules under observation vibrate at a frequency called the resonance frequency. At the resonance frequency, energy is transferred to the molecule, which vibrates more vigorously. This in turn causes the molecule to transfer energy to the surrounding medium, thus increasing the temperature of the medium along with an increase in pressure, in the form of pressure or sound waves, which are detectable by a microphone.

In six flavor facilities, a photoacoustic IR spectrometer was used to collect real-time measurements of airborne diacetyl concentrations.[46] The advantage of this instrument is its portability and it can detect diacetyl vapor in the subpart per million range when utilizing an optical filter with a center wavelength of 11.1 μm. However, the number of chemicals that can be evaluated at a given time is restricted due to use of optical filters.[107] The optical filter only allows specific wavelengths to pass through, thus the photoacoustic IR is selective when target compound has a different wavelength from other compounds in the mixture. However, if the compounds in the mixture share the same wavelength as the target compound, there is no

selectivity. When Martyny et al[46]used the optical filter with a center wavelength of 11.1 μm (wavenumber of 900 cm^{-1}) to monitor diacetyl, if there was 2,3-pentanedione in the air, which also included wavenumber of 900 cm^{-1}, the photoacoustic IR method would not have been selective. To monitor acetoin, diacetyl, and 2,3-pentanedione, bandwidths of the filters can be selected based on the main peaks of IR spectrogram of the ketones provided above.

To measure total organic vapors in air, a direct reading PID is also used in the field.[2] PID has a detection range of 0.2-2000 ppm and is useful for compounds with low ionization potentials, such as ketones, aromatics, alkenes, or amines that are ionized by UV light.[107] Compounds have characteristic ionization potentials and become excited and lose an electron, which forms a positively charged gaseous ion. These ions are collected onto an electrode and the ion current is translated into concentration because the current produced is directly proportional to the mass and concentration. Ionization commences when the UV energy is higher than the ionization potential of the compound and different PID lamps (9.5 eV, 10.0 eV, 10.2 eV, 10.6 eV, 11.7 eV, and 11.8 eV) are available depending on the target chemicals. The proximity of ionization energy of acetoin was 9.4 eV,[112] the diacetyl ionization energy was 9.21 ± 0.05 eV,[113] and the 2,3-pentaneidone adiabatic ionization energy was 9.10 ± 0.04 eV.[114] Thus all of the available lamps would detect acetoin, diacetyl, and2,3-pentanedione, but 9.5 eV gave most sensitivity.

A portable GC is also used in field monitoring because it is suitable for identification of specific chemicals in mixtures and unknown chemicals.[107] Portable GCs are particularly useful for monitoring volatile compounds ranging from 0.1-10,000 ppm. A packed or capillary column is used with the portable GC to separate complex mixtures of gases. After mixture separation,

several possible detectors are available and include flame ionization, electron capture, thermal conductivity, flame photometric, mass spectrometry, and photoionization.

2.7.2 Integrated Sampling Methods with Physisorption

Several air sampling methods for diacetyl, acetoin, and 2,3-pentanedione have been established by NIOSH and OSHA using dynamic sampling tubes involving physisorption. Table 2-4 provides a summary of the current air sampling and analytical methods specifically developed for acetoin, diacetyl, and 2,3-pentanedione.

NIOSH Method 2557[115] and 2558[16] are air sampling and analysis methods for diacetyl and acetoin respectively. Samples are collected by drawing a known volume of air through Anasorb CMS solid sorbent tubes (150/75 mg, 20/40 mesh). Samples are desorbed with 1 mL of acetone: methanol (99:1) and (95:5) for diacetyl and acetoin respectively for 1.5 hours in a rotary mixer. Samples are analyzed by GC-FID. The recommended sampling flow rate and collection volume are 0.01 to 0.2 L/min and 1 to 10 L for both diacetyl and acetoin. The working range was 0.057 to 13.4 ppm (0.20 to 47.2 mg/m^3) for a 10-L air sample for diacetyl and 0.17 to 21 ppm (0.6 to 75.6 mg/m^3) for a 5 L air sample for acetoin. However, NIOSH Method 2557 is affected by high humidity, diacetyl concentration, and days of sample storage prior to extraction, which can cause breakthrough and lead to underestimation of actual diacetyl concentrations.[46, 63, 115] Thus, this method is no longer recommended.[116] In a study, the association between 5.0 ppm diacetyl recovery and absolute humidity was expressed sigmoidally, and diacetyl recoveries were less than 30 and 50 % when absolute humidities were above 10 and 8 mg of water per liter of air sampled respectively.[63] Therefore, a mathematical correction procedure[63] has been used to estimate true diacetyl concentration.

OSHA Method PV2118[117] is an air sampling and analysis method for diacetyl. Samples are collected by drawing a known volume of air through two silica gel sampling tubes connected in series (150/75 mg, 20/40 mesh). Samples are extracted with 1.0 mL of ethyl alcohol: water (95:5) and analyzed by GC-FID. In this method, the target concentration is 25 ppm (88 mg/m^3), and the RQL is 0.28 ppm (1.00 mg/m^3). The recommended sampling time and sampling rate are 60 min at 0.05 L/min (3 L). The extraction efficiency was determined by liquid-spiking silica gel tubes with diacetyl at 0.1 to 2 times 88 mg/m^3, and the recovery over the studied range was 99.1 ± 4.8 %. Since cooking popcorn headspace air concentration is about 0.5-1.0 mg/m^3,[67] the RQL concentration of 1.00 mg/m^3 is not low enough if consumer exposures are to be measured. Modified OSHA Method PV2118 has also been used in some field sampling.[65] This modified method uses 600 mg of silica gel at 0.05 L/min and GC-FID analysis.

OSHA Method 1012[17] is an air sampling and analysis method for diacetyl and acetoin together for monitoring ppb levels. Samples are collected by drawing air through two tubes containing specially cleaned and dried silica gel connected in series (600/600 mg, 20/40 mesh). Samples are extracted and derivatized with a 2.0 mL solution of 95:5 ethyl alcohol:water containing 2 mg/mL of PFBHA and analyzed by GC-ECD. The target air concentrations of acetoin and diacetyl are 50 ppb (0.18 mg/m^3). The RQLs of acetoin and diacetyl are 1.49 ppb (5.37 μg/m^3) and 1.30 ppb (4.57 μg/m^3) respectively. The recommended sampling time and sampling rate for diacetyl and acetoin are 180 min at 0.05 L/min (9.0 L) (TWA) and 15 min at 0.2 L/min (3.0 L) (short term). The mean extraction efficiency recoveries over the studied range were 102.0 ± 1.2 % for acetoin and 97.6 ± 1.4 % for diacetyl. When the samples were stored at 23 °C for 18 days, the recovery of acetoin was above 98.4 % with the overall procedure precision at 95% confidence level of ± 9.9 %. Similarly, the recovery of diacetyl was 98.0 % with overall

procedure precision of ± 10.0 %. Each precision included an additional 5 % variability for the sampling pump. The dsadvantage of this method is that the derivatization step requires 36 hours at room temperature after first rotating samples for 1 hour. This is because this method requires diacetyl to react completely to the di-derivative of PFBHA.

OSHA Method 1013[18] is another air sampling and analysis methods for acetoin and diacetyl together for monitoring low ppm levels. Samples are collected by drawing air through two tubes containing specially cleaned and dried silica gel connected in series (600/600 mg, 20/40 mesh). Samples are extracted with 2.0 mL of ethyl alcohol:water (95:5) and analyzed by GC-FID. The target air concentrations of acetoin and diacetyl are 0.5 ppm (1.8 mg/m^3) and 0.5 ppm (1.78 mg/m^3) respectively. The RQLs of acetoin and diacetyl are 0.011 ppm (0.039 mg/m^3) and 0.012 ppm (0.041 mg/m^3) respectively. The recommended sampling time and sampling rate for acetoin and diacetyl are 180 min at 0.05 L/min (9.0 L) (TWA) and 15 min at 0.2 L/min (3.0 L) (short term). The extraction efficiency was determined by liquid-spiking silica gel tubes, and the recovery over the studied range was 92.9 ± 2.5 % for acetoin and 99.6 ± 3.1 % for diacetyl. If sample concentration was too low to detect by this method, the samples could be derivatized and analyzed by Method 1012.

OSHA Method 1016[23] is an air sampling and analysis methods for 2,3-pentanedione. Samples are collected by drawing air through two tubes containing specially cleaned and dried silica gel connected in series (600/600 mg, 20/40 mesh). Samples are extracted with 2.0 mL of ethyl alcohol:water (95:5) and analyzed by GC-FID. 3-Pentanone was used as an internal standard. The target air concentrations of 2,3-pentanedione is 0.5 ppm (2.05 mg/m^3) with standard error of estimate of 10.1 %. The RQL is 9.3 ppb (38 μg/m^3). The recommended sampling time and sampling rate are 200 min at 50 mL/min (10.0 L) (TWA), and 15 min at 0.2

L/min (3.0 L) (short term). When samples are collected for acetoin and diacetyl along with 2,3-pentanedione, 180 min at 50 mL/min (9.0 L) (TWA), and 15 min at 0.2 L/min (3.0 L) (short term) should be used. The extraction efficiency of 0.1 to 2 times the target concentration was 97.6 ± 1.1%. Recovery at the RQL concentration is 97.9 %, but SD was not provided. When the samples were stored at 4 °C for 17 days, the recovery of 2,3-pentanedione was above 91.3 % with the precision at 95% confidence level of ± 10.1 %, which contained an additional 5 % for sampling pump variation.

To screen VOCs including ketones, NIOSH 2549[118] is also used in field sampling.[2, 7, 65] In this method, samples are collected using a thermal desorption tube, which is a multi-bed sorbent tube containing graphitized carbons and CMS sorbents. The recommended flow rate is 0.01 to 0.05 L/min. A thermal desorption system interfaced to GC-MS is used to analyze samples. This method is used to quantify a wide range of organic compounds based on mass spectral detection. There are more published methods to sample ketones in air, such as, NIOSH method 1300[119], 1301[120], 2553[121], and 2555[122]. However, they were not developed specifically for acetoin, diacetyl, and 2,3-pentanedione sampling and analysis.

Silonite-coated canisters (6L) have also been utilized in field sampling of diacetyl and 2,3-pentanedione vapors.[65] In this method, sampling has been performed at 0.08 L/min for 51 min or 0.02 L/min for 410 min, while external flow controllers regulated air flow into the canisters. Aliquots from the air samples were pre-concentrated in the laboratory, and were analyzed by GC-MS. Since canister samples were analyzed by direct injection into the gas chromatograph, this method was more sensitive than sorbent tube methods which required solvents to desorb ketones prior to injection.[65]

Helium diffusion samplers were developed to eliminate some of the issues with methods that utilize adsorbent media.[123] Traditional active and diffusion samplers have issues with temperature, humidity, face velocity, and chemical matrix effects that can influence uptake rates, back diffusion, and breakthrough. In this method, a small 1" × 4" sampler is worn near the collar during either a 15 min STEL or an 8-hour TWA measurement. Air is collected continuously at a steady rate via a vacuum generated by loss of helium from the device. The sampler is then weighed to determine collected sample weight and the sample is analyzed in a laboratory. Although no details are known, this method is currently being evaluated to sample diacetyl.

Table 2-4: Summary of Acetoin, Diacetyl, and 2,3-Pentanedione Established Sampling and Analytical Methods

Methods	Compounds	Medium	Solvent	Detector
NIOSH 2257	Diacetyl	CMS	Acetone:Methanol (99:1)	GC-FID
	Working range conc. 0.057 to 13.4 ppm			
NIOSH 2558	Acetoin	CMS	Acetone:Methanol (95:5)	GC-FID
	Working range conc. 0.17 to 21 ppm			
OSHA PV2118	Diacetyl	Silica Gel	Ethanol:Water (95:5)	GC-FID
	RQL 0.28 ppm; Target conc. 25 ppm			
OSHA 1012	Acetoin Diacetyl	Silica Gel	Ethanol:Water (95:5) containing 2 mg/mL of PFBHA	GC-ECD
	Acetoin RQL 1.49 ppb; Target conc. 50 ppb Diacetyl RQL 1.30 ppb; Target conc. 50 ppb			
OSHA 1013	Acetoin Diacetyl	Silica Gel	Ethanol:Water (95:5)	GC-FID
	Acetoin RQL 11 ppb; Target conc. 0.5 ppm Diacetyl RQL 12 ppb; Target conc. 0.5 ppm			
OSHA 1016	2,3-Pentanedione	Silica Gel	Ethanol:Water (95:5)	GC-FID
	RQL 9.3 ppb; Target conc. 0.5 ppm			

2.7.3 Integrated Sampling Methods with Chemisorption

Chemisorption methods are better for reactive and very volatile compounds because once a compound reacts with the sampling medium, the derivatives are less volatile. Thus,

chemisorption methods result in derivatives; whereas, physisorption methods may allow escape of volatiles. Because acetoin, diacetyl, and 2,3-pentanedione are ketones, which contain carbonyl functional groups, o-phenylenediamine, DNPH, and PFBHA are used to coat solid sorbents. Absorption by reaction with liquid solutions in impingers is also possible.

NIOSH is developing a sampling and analytical method for vapors of diacetyl and other α-dicarbonyl compounds based on derivatization with the reagent o-phenylenediamine.[124] As shown in Figure 2-1, o-phenylenediamine reacts with α-dicarbonyl compounds, forming quinoxalines and water.[125]

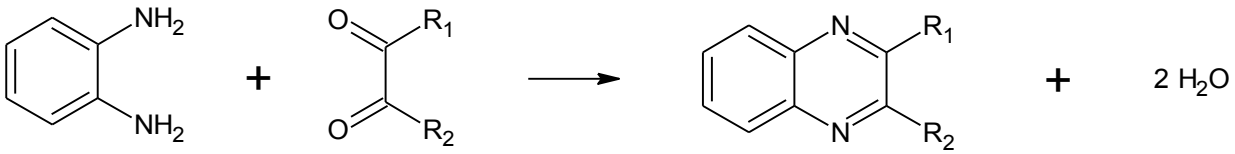
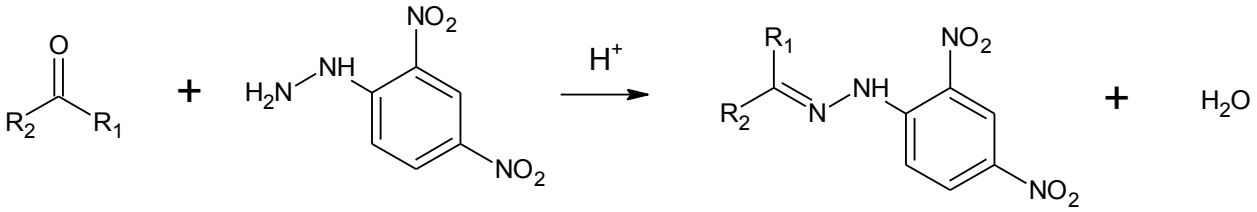


Figure 2-1: Reaction of o-Phenylenediamine and α-Dicarbonyl Compounds

NIOSH Draft Procedure SMP2 was used to measure ketones (diacetyl, 2,3-pentanedione, 2,3-hexanedione, 2,3-heptanedione) in air from five buttermilk flavorings from five different flavorings manufacturers.[65] In this method, samples are collected by drawing a known volume of air through sampling tubes (600/600 mg, mesh size not provided) containing silica gel coated with o-phenylenediamine. Air was sampled at 0.15 L/min flow rate for 53 min or 0.05 L/min for 53-263 min. To analyze the sample, gas chromatography-nitrogen phosphorus detection was used. This method was about three times more sensitive for 2,3-pentanedione than OSHA method 1013.[65] The o-phenylenediamine reaction was also used to determine diacetyl in foods and beverages, such as beer, brandy, vinegar, wine, and butter samples,[125, 126] and in cigarette smoke[36] by detecting the derivative, 2,3-dimethylquinoxaline.

Another chemisorption method is developed using DNPH. EPA TO-5 is one such method to determine aldehydes and ketones in ambient air.[127] In this method, ambient air is drawn at 100-1000mL/min through a midget impinger containing 10 mL of 2N HCl/0.05 % DNPH and 10 mL of isooctane. The function of the isooctane layer is to extract DNPH derivatives. Figure 2-2 shows the reaction of carbonyl compounds and DNPH, which forms a stable colored hydrazone derivative.



carbonyl group DNPH stable color hydrazone derivative water

R_1 = H, alkyl, phenyl and R_2 = H, alkyl, phenyl

Figure 2-2: Reaction of Carbonyl Compounds (Aldehydes and Ketones) and DNPH

To recover DNPH derivatives, the isooctane layer is removed, the aqueous layer is extracted with 10 mL of 70/30 hexane/methylene chloride, and the organic layers are combined. The combined organic layers are evaporated by a nitrogen stream and sensitivity is achieved by dissolving the residue in methanol. The DNPH derivatives are analyzed using reverse phase HPLC with a UV detector at 370 nm. Since this method was developed to determined aldehydes and ketones in general, reaction efficiency and recovery were not provided specifically for acetoin, diacetyl, and 2,3-pentanedione.

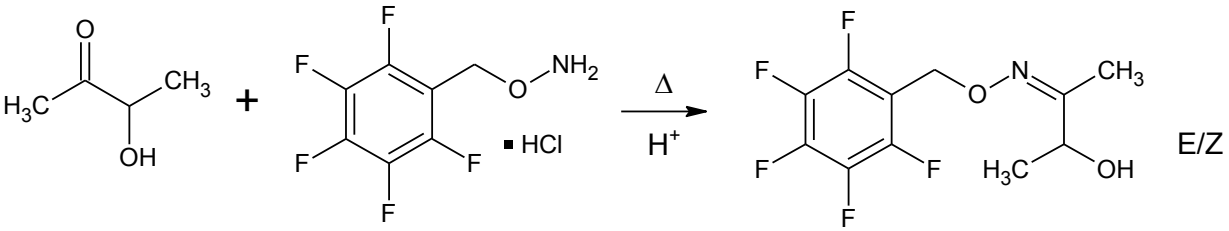
EPA TO-11A is another method for the determination of formaldehyde and other carbonyl compounds (aldehydes and ketones) in ambient air, and a known volume of air is drawn through a pre-packed silica gel cartridge coated with acidified DNPH at a sampling rate of 100-2000 mL/min.[128] The DNPH derivatives are analyzed by HPLC with a UV absorption detector

operated at 360 nm. EPA Method IP-6A for active sampling also employs DNPH-coated silica gel adsorbent tubes and utilizes HPLC for analysis.[69] EPA Method IP-6C is used for passive sampling and involves the same chemisorption sampling with HPLC analysis.[129] The passive sampler contains silica gel filter paper treated with DNPH. Overall, the DNPH chemisorption method followed by HPLC analysis is applied to different sampling methods. For example, a cryotrapping method was developed to concentrate carbonyl compounds using glass traps cooled in liquid nitrogen.[130] The DNPH method has actually been used to monitor diacetyl exposure in the field, and samples were analyzed by GC.[6]

The DNPH method is useful to detect aldehydes and ketones. However, there are potential problems due to different ketones forming the sample derivatives. For instance, OSHA unsuccessfully attempted this method to sample diacetyl and acetoin.[17] DNPH reacted with acetoin and diacetyl forming unique derivatives, but it subsequently reacted with the alcohol group on acetoin and the second ketone group on diacetyl, which resulted in the same derivative. Therefore, this method has potential problems where α-diketones and α-hydroxy ketones exist simultaneously in air.

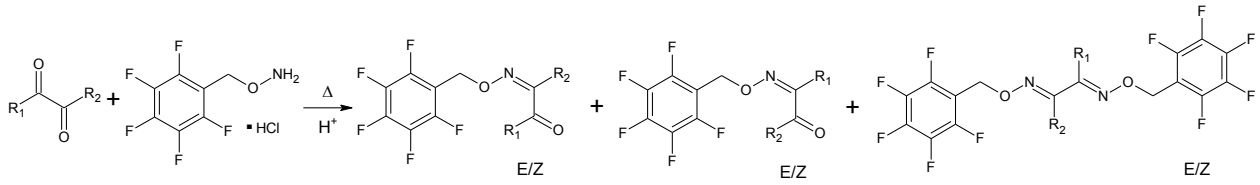
To solve the derivative issue, reaction with PFBHA can distinguish between diacetyl and acetoin.[17] The reaction of PFBHA with aldehydes and ketones forms O-oximes, which allows quantification of carbonyl compounds.[17, 131] Other advantages of PFBHA are its fast quantitative reaction in water to form O-oximes and its suitability for detection at the pg level by GC-MS using SIM of mass-to-charge ratio (m/z) 181 as well as GC-ECD.[131] For example, through reaction with PFBHA, carbonyl compounds including diacetyl have been identified in various samples, such as in environmental samples, in PM emitted from vehicles, and in drinking

water as ozone disinfection byproducts.[33, 35, 132-134] Figure 2-3 and Figure 2-4 show the reaction of acetoin, diacetyl, and 2,3-pentanedione with PFBHA to form E- and Z-isomers of O-oximes.



acetoin PFBHA O-oxime

Figure 2-3: Reaction of Acetoin and PFBHA



α-diketone PFBHA mono-substituted O-oximes di-substituted O-oxime

R_1 is CH_3 in diacetyl and 2,3-pentanedione

R_2 is CH_3 in diacetyl and CH_2CH_3 in 2,3-pentanedione

Figure 2-4: Reaction of Diacetyl or 2,3-Pentanedione and PFBHA

As previously mentioned, OSHA 1012 uses physisorption with silica gel, then diacetyl and acetoin are derivatized with PFBHA. However, since acetoin can be converted to diacetyl and vice versa during sampling and analysis,[16] conversion may occur before the compounds are derivatized. Also, acetoin, diacetyl and 2,3-pentanedione are light sensitive and decompose even after becoming trapped in the sorbent tubes.[17, 23] Thus, a chemisorption method can prevent this issue if PFBHA is first coated onto the solid sorbent because it will allow on-tube derivatization. The collection efficiency of a denuder-filter sampling PFBHA coated and uncoated XAD-4 resin has been compared. Specifically for diacetyl, the collection efficiency of the denuder coated with XAD-4 was 29.6 % for a sampling time of 10 min, but XAD-4 coated with PFBHA was

88.9 % with no provided SDs.[134] Thus, the solid sorbent should be coated by PFBHA in order to maximize the recovery potential of carbonyl compounds.

In order to use PFBHA in dynamic sampling or passive sampling, solid sorbents should be used for coating. However, many different kinds of solid sorbents are commercially available. Thus, the best solid sorbent has to be selected in order to perform selective and sensitive air sampling. Wu et al. attempted to identify the most appropriate solid sorbents to coat with 20 % weight per weight (w/w) PFBHA.[135] The solid sorbents that Wu et al. tested were silica gel (20/40 mesh) in silica gel tubes, XAD-2 (20/60 mesh), XAD-7 (20/60 mesh), Florisil (60/100 mesh), Porapak Q (50/80 mesh), Tenax GC (80/100 mesh), Chromosorb 101 (80/100 mesh), Chromosorb 102 (80/100 mesh), and Chromosorb 106 (60/80 mesh). Tenax GC has been replaced by Tenax TA commercially.

After solid sorbents were coated with PFBHA, visual inspection and weighing showed that silica gel, XAD-2, XAD-7, and Porapak Q did not coat evenly.[135] Then, 1 μL of valeraldehyde was spiked onto 200 mg of the coated solid sorbent tubes that were evenly coated. Coated Florisil, Chromosorb 101, and Chomosorb 106 were excluded from further testing because PFBHA O-oxime recoveries were lower than 80 %. Vapor/solid sorbent reaction efficiency was then determined by dynamic air sampling using valeraldehyde vapor at a RH of 1 % and 90 %. Both Tenax GC and Chromosorb 102 gave equivalent recoveries of about 100 % at RH of 1 % and 90 %, but precisions were better for Tenax GC (CVs 5-6 %) relative to Chromosorb 102 (CVs 11-12 %). Thus, Tenax TA was used as a solid sorbent to coat with PFBHA.

Furthermore, Tenax TA was tested for its capacity to trap water vapor, and results showed that water uptake rates were below the sensitivity of a water sampling procedure.[136]

The water saturation capacity of Tenax TA (mesh 20/35) was < 3.3 mg of H_2O/g of material in the ranges 10-40 ºC and 20-100 % RH. Thus, Tenax TA is suitable for sample collection under all humidity conditions, which helps to sample artificial butter flavorings during hot processes, or when high humidity conditions are present.

The PFBHA coated Tenax TA method seems to have advantages. The PFBHA coated Tenax TA method can be used for both dynamic and passive sampling for aldehydes and ketones.[135, 137-142] There were no effects from humidity and temperature, and PFBHA derivatives of aldehydes and ketones can be quantified at the pg level by GC-MS. The method allows quantification of aldehydes and ketones accurately and precisely, if they are not sterically hindered.

When chloroacetone, cyclohexanone, diacetone alcohol, diethyl ketone, dipropyl ketone, ethyl butyl ketone, methyl amyl ketone, methyl butyl ketone, 2-methylcyclohexanone, methyl ethyl ketone, methyl isobutyl ketone, methyl isopropyl ketone, and methyl propyl ketone were sampled with 200-mg 20 % w/w PFBHA on Tenax TA, gas phase recoveries up to 200 ppm-hour exceeded 75 % by desorption of the sorbent with 3 mL hexane by 2 min manual agitation at 25 °C.[142] However, the recoveries for acetophenone, 2-chloroacetophenone, and ethyl amyl ketone vapor were lower than 75% although ketone PFBHA O-oxime yields for synthesis and liquid spiking recoveries of ketones were above 90%. An inhibition of the gas/solid phase reaction relative to the efficient liquid/solid phase and liquid phase reaction was thought due to steric hindrance and restriction of access to the carbonyl group by the alkyl groups. The results indicated that even if vapor/solid reaction did not occur completely on the tube, if ketones adsorb on the tube, they may react in aqueous solution during desorption.

An advantage for passive sampling is that the samplers do not require a pump which is useful for personal monitoring because the monitor can be easily attached to the collar of a worker's clothes. However, all OSHA and NIOSH methods listed above are dynamic air sampling methods. OSHA 1012 and 1013 methods require the use of bulky sorbent tubes with operation of a pump, which is inconvenient for workers. Thus, an air sampling method that is not affected by temperature and RH and that can detect low concentration with a light sampler should be developed to help quantify the concentration of acetoin, diacetyl, and 2,3-pentanedione in the workplace.

2.8 Diffusion Theory and Adsorption for Passive Sampling

To determine theoretical sampling constants of acetoin, diacetyl, and 2,3-pentanedione, the Fuller-Schettler-Giddings method and Fick's First Law of Diffusion are used to calculate the theoretical sampling constant in this research. The equation from Fuller-Schettler-Giddings method was used to calculate the dependence of the diffusion constant on molecular weight and temperature (equation 2.1):[143, 144]

$$D_{AB} = \frac{0.00143T^{1.75}}{P_{ext}M_{AB}^{0.5}\left((\Sigma_v)_A^{1/3} + (\Sigma_v)_B^{1/3}\right)^2} \quad (2.1)$$

where D_{AB} is the binary diffusion coefficient of analyte in air in cm^2/sec at T; T is temperature in Kelvin; $M_{AB} = 2[(1/M_A)+(1/M_B)]^{-1}$; M is molecular weight in g/mol; A is air; B is the analyte (acetoin, diacetyl or 2,3-pentanedione); P_{ext} is the external pressure in bar; Σv is the summation of atomic diffusion volumes, unitless.

The molecular diffusion volume of air is 19.7.[144] The molecular diffusion volume of acetoin ($C_4H_8O_2$), diacetyl ($C_4H_6O_2$), and 2,3-pentanedione ($C_5H_8O_2$) is calculated using equation 2.2, 2.3, and 2.4 respectively:

$$(\Sigma_v)_{acetoin} = 4 \times 15.9^{C} + 8 \times 2.31^{H} + 2 \times 6.11^{O} = 94.3 \quad (2.2)$$

$$(\Sigma_v)_{diacetyl} = 4 \times 15.9^{C} + 6 \times 2.31^{H} + 2 \times 6.11^{O} = 89.7 \quad (2.3)$$

$$(\Sigma_v)_{2,3\text{-pentanedione}} = 5 \times 15.9^{C} + 8 \times 2.31^{H} + 2 \times 6.11^{O} = 110.2 \quad (2.4)$$

where the atomic diffusion volume increment for carbon, hydrogen, and oxygen is C = 15.9, H = 2.31, and O = 6.11 respectively.[144]

The diffusion coefficients of acetoin, diacetyl, and 2,3-pentanedione at 25ºC and 1 atm (1.01 bar) are 0.087, 0.089, and 0.080 respectively as shown in equation 2.5, 2.6, and 2.7 respectively:

$$D_{air-acetoin} = \frac{0.00143 \times 298^{1.75}}{1.01 \times \left[2\left(\frac{1}{29} + \frac{1}{88.1}\right)^{-1}\right]^{1/2} \times \left[19.7^{1/3} + 94.3^{1/3}\right]^2} = 0.087 \text{ cm}^2/\text{s} \quad (2.5)$$

$$D_{air-diacetyl} = \frac{0.00143 \times 298^{1.75}}{1.01 \times \left[2\left(\frac{1}{29} + \frac{1}{86.1}\right)^{-1}\right]^{1/2} \times \left[19.7^{1/3} + 89.7^{1/3}\right]^2} = 0.089 \text{ cm}^2/\text{s} \quad (2.6)$$

$$D_{air-2,3-pentanetione} = \frac{0.00143 \times 298^{1.75}}{1.01 \times \left[2\left(\frac{1}{29} + \frac{1}{100.1}\right)^{-1}\right]^{1/2} \times \left[19.7^{1/3} + 110.2^{1/3}\right]^2} = 0.080 \text{ cm}^2/\text{s} \quad (2.7)$$

For a cylindrical open tube, a theoretical sampling constant k is defined through Fick's First Law of Diffusion as shown in equation 2.8:[137, 140, 141, 145]

$$\frac{dm}{dt} = \left(\frac{D_{AB}A}{L}\right)\left(c_{air} - c_{surf}\right) = k\left(c_{air} - c_{surf}\right) \quad (2.8)$$

where dm/dt is the steady state mass sampling rate or mass transfer rate weight/time; A is the cross-sectional area of the sampling surface in cm^2, L is the diffusion path length in cm; c_{air} is the air concentration of the analyte in weight/cm^3; c_{surf} is the air concentration of analyte just above

the sampling surface in weight/cm^3; $k = D_{AB}A/L$ is the sampling constant of the analyte in cm^3/time, the parameter to be determined for field passive sampling.

3 Methods

This section outlines the materials and equipment used to perform the experiments. The detailed procedures involved in the development of dynamic and passive air sampling methods for acetoin, diacetyl, and 2,3-pentanedione are also provided.

3.1 Materials

PFBHA (99+%) was obtained from Fluka (Milwaukee, WI), Alfa Aesar (Ward Hill, MA), and Lancaster Laboratories Inc. (Lancaster PA). Tenax TA (80/100 mesh) was from Grace (Deerfield, IL). The ketones investigated were acetoin (99.9%) from Supelco (St. Louis, MO); diacetyl (99+%) from Fluka; and 2,3-pentanedione (97%) from Sigma-Aldrich (Milwaukee, WI). n-Heptanal from Alfa Aesar was used to synthesize the internal standard, the n-heptanal PFBHA O-oxime. Ethanol (95%, 190 proof), DBP (99%), and 1,4-dioxane (99%) were from Acros Organics (Morris Plains, NJ). Hexanes (Optima), methanol (Optima), acetonitrile (Optima), chloroform (Optima), methylene chloride (Optima), tetrahydrofuran (Optima), cyclohexane (HPLC), nitromethane (certified ACS), pentane (certified), nitric acid (certified ACS), lithium chloride, calcium chloride, magnesium chloride, potassium carbonate, sodium dichromate, sodium chloride, potassium chloride, potassium nitrate, boiling chips, Drierite with indicator, and Snoop liquid leak detector were from Fisher Scientific (Pittsburgh, PA). Helium (99.999%), nitrogen (99.999%), and dry grade air were from Air Liquide (Plumsteadville, PA).

3.2 Equipment

The following were purchased from Fisher Scientific: Pyrex glass tubes, 5-mm ID and 7-mm OD cut into lengths of 7 cm with ends fire-polished;[138] Pyrex glass wool; aluminum foil;

spatulas; weighing paper; 4-mL amber and Pyrex vials with PTFE-lined screw caps; 5-mL V-vials, BD Falcon 15-mL polypropylene conical centrifuge tubes; 5-mL and 10-mL Pyrex ground glass volumetric flasks; 50-mL and 100-mL beakers; 250-mL glass jars with PTFE lined polypropylene caps; 500-mL round bottom flasks; Pasteur pipettes; 10-100, 100-1000, and 500-5000 µL Eppendorf adjustable volume pipettes; 10-µL Hamilton syringes; 5-mL and 50-mL gastight Hamilton syringes; stands; Soxhlett extraction apparatus and Whatman Soxhlett extraction thimbles; Scienceware glove box and gloves; Pyrex vacuum desiccators; Bel-Art Scienceware transparent vacuum desiccator with a ceramic metal plate utilized as exposure chamber; Teflon-lined screw caps 14-mm ID and 16-mm depth; National Scientific 20-mm unlined aluminum crimp seals and crimper, Kontes 3-place gas sampling manifold; Harvard syringe pump Model 11; Teflon tubing 3/16-in ID and 1/4-in OD; Tygon R-3603 tubing 1/4-in ID and 3/8-in OD; Parr 2811 bench manual pellet press; 3M Model 3500 organic vapor monitors; Branson ultrasonic cleaner; Centrific Model 228 centrifuge; Mettler AE260 DeltaRange analytical balance; Thermolyne Series 5000 carbon dioxide incubator; Marathon Management electronic digital micrometer; vernier; disposable nitrile gloves; chemical resistant nitrile gloves.

Water was deionized using a Milli-Q Water System and Simplicity 185 from Millipore (Billerica, MA). A Rotavapor R-3 was from Buchi Corporation (New Castle, DE). M-5 mini-Buck Calibrator was from A.P. BUCK Inc. (Orlando, FL). A DryCal Defender 520 gas flow meter was from Bios International Corporation (Butler, NJ). Climomaster Model A533 (multi-function thermal anemometer) was from Kanomax (Andover, NJ) to measure air temperature, RH, and air velocity. The following were obtained from SKC Inc. (Eighty Four, PA): 25-mL midget impingers; rotameters; 10- to 100-L Tedlar Air Sample Bags; Pocket Pump 210-1102,

Model 224-PCXR4, and Model 224-52 personal sampling pumps. 1875 watt hair dryer Model 127S was from CONAIR Glendale, AZ. Forma Environmental Chamber Model 3940 and alcohol thermometer were from Thermo Scientific (Marietta, Ohio). Economy Oven Model 45EG was from Precision Scientific (Chicago, IL). The microwave oven was from Gold Star USA, Inc. (Englewood cliffs, NJ). A Vibro-Graver vibrator was from Burgess Vibrocrafters (North Adams, MA). 1-1/4 in diameter clear acrylic circles were from United States Plastic Corporation (Lima, OH). The MP15 laboratory pellet press with built-in hydraulic pump and 30-mm ID dry pressing die set were from Across International (Berkeley Heights, NJ). POWERSTAT Variable Transformer 116B was from Superior Electric (Bristol, CT). Heating tapes and Labquake shaker/Rotisserie were from Barnstead Thermolyne (Dubuque, IA). Syringe pump (74900-00-05) was from Cole-Parmer Inc. (Vernon Hills, IL). A small box desk air fan (14244-01) was from Tekna Design, (Rockford, MI). Stainless steel tubing 6.4-mm OD and stainless steel swagelok were from Alltech Associates (Deerfield, IL). Thermogreen septa with extremely low bleed over 100 to 350 °C were from Supelco. A silicone half mask (7700-30M) with 7581P100 cartridge was from NORTH (Cranston, RI)

GC-MS was conducted with a 6890N Network Gas Chromatograph equipped with an HP-5ms fused silica capillary column (30-m length, 0.25-mm ID, and 0.25-µm film thickness) from Agilent Technologies (Santa Clara, CA). It was linked to the 70 eV electron impact ion source of a 5973Network Mass Selective Detector with quadrupole mass spectrometer and an electron multiplier detector. The injector (inlet) temperature was 250 ºC and GC-MS transfer line (auxiliary) was 280 ºC. The MS ion source and quadrupole temperatures were 230 ºC and 150 ºC respectively. The carrier gas for GC-MS was helium and 2-µL hexane solution was injected at 1.0 mL/min in the splitless mode.

3.3 Synthesis of PFBHA O-Oximes

The acetoin, diacetyl, 2,3-pentanedione, and n-heptanal PFBHA O-oximes were first synthesized because PFBHA O-oximes for the ketones were not commercially available. Since diacetyl and 2,3-pentanedione had two carbonyl groups, the mono- and di- substituted PFBHA O-oximes of diacetyl (M-diacetyl and D-diacetyl) and the mono- and di- substituted PFBHA O-oximes of 2,3-pentanedione (M-2,3-pentanedione and D-2,3-pentanedione) were synthesized to assess the recovery after chemisorption of ketones onto the PFBHA coated Tenax TA. Since acetoin and n-heptanal had one carbonyl group, only the mono-substituted PFBHA O-oximes of acetoin and n-heptanal (M-acetoin and M-heptanal) were synthesized.

To synthesize the mono-substituted PFBHA O-oxime of monocarbonyl compounds (M-acetoin and M-heptanal), a 1:1 molar ratio of PFBHA:ketone was used. To synthesize the mono-substituted PFBHA O-oxime of dicarbonyl compounds (M-diacetyl and M-2,3-pentanedione), a 1:1.2 molar ratio of PFBHA:ketone was used to prevent production of di-substituted PFBHA O-oximes by adding a slight excess of the ketones. To synthesize the di-substituted PFBHA O-oxime of dicarbonyl compounds (D-diacetyl and D-2,3-pentanedione), 2.3:1 and 2.5:1 molar ratios of PFBHA:ketone were used.

All glassware was washed with detergent, rinsed with distilled water, soaked at least 24 hours in a 10 % nitric acid bath, rinsed in distilled water, and dried in an oven at 100 ºC. Chemical resistant gloves were worn over disposable nitrile gloves when placing and removing glassware from the acid bath. During all other operations, only disposable nitrile gloves were worn.

To synthesize 100 mg of their PFBHA O-oxime derivatives, the ketones were dissolved in 3 mL of distilled water in 4-mL amber vials. The ketone solution was added into an aqueous

solution containing PFBHA in 3 mL of distilled water in a 15-mL centrifuge tube. The solution was vigorously shaken for 1 min, ultrasonicated for 2 min, and placed in a microwave oven until the first bubbles appeared in the aqueous solution. The first bubble appeared after about 10 sec of heating and indicated that the solution had reached about 80 ºC. However, the temperature of the solution was not determined in each experiment to minimize the loss of the products. The solution was cooled at room temperature for 1 hour, 2 days, or 5 days depending on the synthesis as shown in Table 4-1. For 2 days and 5 days synthesis, a shaker at 30 rpm was used to mix the solution. Then, 4 mL of hexane were added to the aqueous solution, and the tube was manually shaken for 1 min and centrifuged at 1500 rpm for 20 min to separate the two layers. The hexane layer was transferred into a V-vial. The extraction procedure was repeated two more times, and all of the hexane solution was combined and evaporated at room temperature under a nitrogen gas stream. The vial contents were further dried in a vacuum desiccator until constant weight was attained, so that the yield could be calculated.

The yield of PFBHA O-oxime product was determined from the dry weight based on the assumed 1:1 stoichiometry for the mono-substituted PFBHA O-oxime and 1:2 stoichiometry for the di-substituted PFBHA O-oximes. Each derivative in hexane at 1 μg/μL was injected into the GC-MS to investigate its purity in the TIC mode as detailed in Section 3.4. TIC analysis was used because it could detect the presence of pentafluorobenzaldehyde, pentafluorobenzyl alcohol, pentafluoroanisole, excess PFBHA, other aldehydes and ketones, and other peaks not attributable to the reagents and solvents.[146] E- and Z-isomers of the AUC were summed to find the purities. Each PFBHA O-oxime was synthesized and analyzed in triplicate. Areas contained in the appropriate method blank were subtracted if interfering, or ignored if not.

3.4 GC Temperature Program Optimization

A GC temperature program was optimized to separate the target PFBHA O-oximes as well as the internal standards. The retention times and mass spectra of the target PFBHA O-oximes were first identified for qualitative analysis. TIC analysis from m/z 50-500 allowed identification of peaks including PFBHA O-oximes and impurities. The column temperature program with TIC used to find the purity of PFBHA O-oxime standards was: solvent delay for 4 min at 50 ºC holding there for 3 min, 20 ºC/min, and 250 ºC holding there for 3 min. Thus, the total program was 16 min while the flow rate was 1 mL/min. This temperature program was set to start from relatively low temperature (50 ºC) to capture impurities such as un-reacted PFBHA and formaldehyde PFBHA O-oxime.

SIM with m/z 121, 181, 239, and 240 allowed sensitive quantitation to the pg level. These ions were selected because all PFBHA O-oximes had m/z 181 as the base peak, DBP had m/z 121 as the base peak, M-heptanal had m/z 239 as the second largest peak, and M-acetoin had m/z 240 as the second largest peak. Since the target concentrations for the ketones were low, SIM was used in this research for quantification of samples. The final optimized temperature program with SIM at m/z 181, 239, and 240 to quantify PFBHA O-oximes was: solvent delay for 4 min with 110 ºC holding there for 1 min, 10 ºC/min, and 250 ºC holding there for 3 min. Thus, the total program was 18 min, while flow rate was 1 mL/min. Both E- and Z- isomer peak areas were used for quantification with an internal standard method. Since this program was created to quantify the target ketone PFBHA O-oximes, the PFBHA peak did not appear on the chromatogram as its retention time was during the solvent delay. This was a compromise to make the runs as short as possible.

3.5 Selection of Internal Standard

DBP was initially used as an internal standard since it was also utilized to analyze aldehydes in air and water by the PFBHA solid sorbent method.[147] DBP had advantages as an internal standard. It was commercially available and did not react with PFBHA. n-Heptanal PFBHA O-oxime was also tested as an internal standard after derivatization of n-heptanal with PFBHA. Assessment of purity involved determination of whether un-reacted PFBHA or n-heptanal remained.

Because samples were usually injected throughout the day and GC-MS conditions changed over time, various chemicals were injected over 8.5 hours. In order to determine which internal standard was suitable for the target PFBHA O-oximes, M-diacetyl, D-diacetyl, DBP, and M-heptanal were combined together in hexane within their liner range concentrations, and 2 μL of the solution was injected into the GC-MS. The concentrations of the samples varied between chemicals, but were consistent for the specific chemical over the 8.5 hours. In this analysis, SIM of m/z 121 for DBP and 181 for the PFBHA O-oximes were used, and the AUC was obtained for each compound.

3.6 E- and Z- Isomer Ratios of PFBHA O-Oximes

Since there were E- and Z- isomers of the PFBHA O-oximes, the largest peak among each O-oxime was used for quantification to simplify the analysis in the preliminary study. However, further investigation was performed at a later time to confirm whether it was sufficient to use only the largest peak for each PFBHA O-oxime.

The AUCs for each D-diacetyl isomer were used to find the corresponding isomer AUC ratios, with the total area normalized to one. This analysis was carried out assuming an equal

GC-MS response factor of isomers. The AUC ratios of D-diacetyl E- and Z- isomers were compared for different conditions: Tenax-water-hexane standard; O-oxime liquid/solid spiking; ketone liquid/solid spiking; ketone vapor/solid sampling at 10 ppb, 25 ºC, and 5 % RH sampled at 100 mL/min for 8 hours. The detailed procedures of standards and spiking are discussed in Sections 3.8 and 3.11 respectively. The D-diacetyl concentration used for the standard was 2.8 µg/mL. O-oxime and ketone concentration spiked on the solid sorbents was equivalent to 10 ppb vapor sampled at 100 mL/min for 8 hours. The Tenax-water-hexane desorption method was used for all conditions. At each condition, triplicate samples were analyzed, and the interrun means, SDs, and CVs were compared. To determine whether means were statistically different, a two-tailed Student t-test was performed assuming unequal variance at $\alpha = 0.05$ and p-values were calculated.

Furthermore, E- and Z- isomer ratios of D-diacetyl were compared to those obtained when diacetyl reacted with PFBHA in aqueous solution or 95:5 ethanol:water solution as performed in OSHA Method 1012[17] by ketone liquid/liquid spiking. Acetoin, diacetyl, and 2,3-pentaedione in water solution were spiked individually and as a mixture into 2 mL of 95:5 ethanol:water solution containing 2 mg/mL PFBHA and set at room temperature for 36 hours as explained in OSHA Method 1012. Then, an aliquot of the solution was injected into a GC-MS instead of GC-ECD as explained in OSHA Method 1012.

3.7 Selection of m/z in SIM

M-heptanal, M-acetoin, M-diacetyl, M-2,3-pentanedione, D-diacetyl, and D-2,3-pentanedione all had a base peak of m/z 181 compared with the DBP internal standard m/z of 121. Thus, it was best if all PFBHA O-oximes, including the internal standard, were completely

separated using SIM with m/z 181 in order to quantify accurately and precisely. However, it was difficult to separate E- and Z-isomers of M-acetoin and M-2,3-pentanedione with acceptable resolution. Thus, it was necessary to identify a large unique m/z, which would be present only in the M-acetoin or the M-2,3-pentanedione mass spectrum. M-acetoin had m/z 240, which was the 2nd largest m/z, but 2,3-pentanedione had no m/z 240 in its mass spectrum at the target concentration range. Thus, SIM with m/z 181 and 240 together was used to determine whether it was possible to quantify a mixture of acetoin and 2,3-pentanedione PFBHA O-oxime congeners.

Known concentrations of M-acetoin were spiked into the three different types of solution to make standard curves (pure hexane, Tenax-hexane, and Tenax-water-hexane) with details described in Section 3.8. The purpose of this experiment was to determine whether the sum of the extracted AUC at m/z 181 and the extracted AUC at m/z 240 (calculated AUC 181 + 240) was statistically the same as the observed AUC obtained with SIM at m/z 181 and 240 as M-acetoin mass injected into GC-MS increased. Thus, the % ratio of calculated to observed AUC was obtained (% Calc/Obs AUC 181+240). Also, the % ratio of extracted AUC at m/z 240 to observed AUC at m/z 181 and 240 was obtained (% Extracted AUC 240).

To quantify the AUC of M-2,3-pentanedione, the combined AUC of M-acetoin and M-2,3-pentanedione peaks was first obtained by SIM with m/z 181 and 240 together. Then, the AUC of M-acetoin alone with m/z 181 and 240 was calculated by using the % extracted AUC 240. After that, the AUC of M-2,3-pentanedione was calculated by subtracting the AUC of M-acetoin from the combined AUC of M-acetoin and M-2,3-pentanedione peaks. In order to find % extracted AUC 240, injection of M-acetoin alone was required before injection of standard curves for mixtures.

Similarly, m/z 239 was added to the SIM optimized temperature program because M-heptanal had m/z 239, which was the 2nd largest m/z. Although the M-heptanal peak had no interference with target PFBHA O-oximes, there were minor background peaks around the retention time, especially for passive sampler method blanks. Thus, known concentrations of M-heptanal were injected into the GC-MS to create pure hexane external standard where there is no background peaks in the solvent blank. Then, it was determine whether the % ratio of extracted AUC at m/z 239 to observed AUC at m/z 181, 239, and 240 (% Extracted AUC 239) remain constant around the concentration used as the internal standard. In order to find % extracted AUC 239, M-heptanal alone in hexane solution (2.5 μg/mL) was injected daily.

3.8 Quantification of PFBHA O-Oximes Using Different Standard Curves

After the temperature programs for SIM mentioned in Section 3.4 were optimized to separate the PFBHA O-oximes, their linear ranges were obtained for quantitation of absolute sampling efficiencies of the original ketones. The synthesized PFBHA O-oximes were used to make standard curves, both external and internal standards, by simple linear regression to produce slopes and intercepts with their SDs, the square of the correlation coefficient (R^2), and p-values. The response factors for the linear ranges of each PFBHA O-oxime were the slopes of the external standard curves. Then, internal standard methods using both DBP and M-heptanal were constructed. The LQLs were also determined from CV values being less than 10 % at the specific concentration.

3.8.1 Internal Standards in Pure Hexane

The dry weight of the synthesized PFBHA O-oxime was measured in a volumetric flask and then dissolved in hexane with shaking. Different concentrations of standard solutions were

prepared by diluting with hexane from concentrates of 1 mg/mL of PFBHA O-oxime in hexane. Standard solutions were prepared for each O-oxime or all O-oximes combined as a mixture with the internal standard. An aliquot (2 μL) in hexane solution was injected into GC-MS for both external and internal standard analysis. This method was used to generate "pure hexane" standard curves. For an external standard, O-oxime mass injected (ng) into GC-MS, after correcting based on the purity of each O-oxime, was plotted on the x-axis and AUC from the GC chromatogram was plotted on the y-axis. For an internal standard, O-oxime mass injected (ng) into GC-MS was plotted on the x-axis and AUC ratio of O-oxime over the AUC of internal standard was plotted on the y-axis.

3.8.2 Internal Standards in Tenax-Hexane

The hexane method blank was used to generate a standard curve and to simulate the actual vapor sample analysis. 200 mg of the PFBHA coated Tenax TA (as detailed in Section 3.10) was suspended in 2 mL of hexane in a 15-mL centrifuge tube. The tube was manually shaken vigorously for 1 min, placed in an ultrasonicator for 2 min, set at room temperature for 1 hour, and centrifuged for 20 min. This hexane solution was used to dilute the O-oxime standard instead of pure hexane. Different concentrations of each O-oxime or O-oxime mixture and 0.1 mg/mL of the internal standard were first prepared using pure hexane. Then, 5 μL of O-oxime solution and 5 μL of internal standard solution were spiked into 190 μL of the treated PFBHA coated Tenax TA hexane solution, and the solution was shaken manually. An aliquot (2 μL) was then injected into the GC-MS for internal standard analysis. This method was used to generate "Tenax-hexane" standard curves.

3.8.3 Internal Standards in Tenax-Water-Hexane

Similarly, 200 mg (dynamic sampling) or 300 mg (passive sampling) of the PFBHA coated Tenax TA was suspended in 2 mL of water in a 15-mL centrifuge tube. The tube was manually shaken vigorously for 1 min, placed in an ultrasonicator for 2 min, heated in a microwave oven until the first bubbles appeared in the aqueous solution, and set at room temperature for 1 hour. A volume of 2 mL of hexane was added into the aqueous solution and the water/hexane solution was shaken for 1 min and centrifuged for 20 min. The extracted hexane solution was used to dilute O-oximes, and the same method described for "Tenax-hexane" standard curves was utilized to generate "Tenax-water-hexane" standard curves.

3.8.4 Reaction of Mono- to Di-Derivatives in the Presence of Excess PFBHA

To determine how much M-diacetyl and M-2,3-pentanedione would turn into di-substituted O-oximes, M-diacetyl or M-2,3-pentanedione alone was spiked into Tenax-hexane or Tenax-water-hexane standard solution. The amount of di-substituted O-oximes found in Tenax-hexane and Tenax-water-hexane standard solutions were determined from the GC-MS chromatograms.

3.8.5 Vapor Phase Sampling

To determine actual ketone concentrations in air, Tenax-water-hexane internal standard curves for each O-oxime were first used to calculate the mass of O-oxime injected in GC-MS. Then, the concentrations of O-oximes in 2 mL of hexane solution were calculated, and the molecular weight ratio of each O-oxime to its original ketone was used to calculate the mass of ketone sampled on the solid sorbents in the dynamic sampling tubes or passive sampling pellets. For diacetyl and 2,3-pentanedione, the ketone masses calculated from both the mono- and di-

substituted O-oximes were summed to quantify the original ketone mass for both ketones. Knowing the mass of ketone and air volume collected in the dynamic sampling tubes or sampling constant for the passive samplers, the concentration in air was determined. The temperature and pressure were observed to allow adjustment to 25 ºC and 760 mm Hg.

3.9 Purification of PFBHA

Because PFBHA reacts with aldehydes and ketones in air, it must be checked for purity by GC-MS analysis. When PFBHA impurities appeared with greater LQLs than the target O-oximes at their retention times, purification of PFBHA was necessary.

In order to purify PFBHA, impurities were extracted by hexane in a nitrogen gas environment to minimize the exposure to aldehydes and ketones in the laboratory air. 300 mg of PFBHA was dissolved in 4 mL of water in a 15-mL centrifuge tube, and the solution was vigorously shaken for 1 min. The tube was placed in a microwave oven until the first bubbles appeared in the aqueous solution. The solution was cooled to room temperature in a glove box filled with nitrogen gas. Then, 4 mL of hexane was added into the aqueous solution, the tube was manually shaken for 1 min, and the hexane layer with impurities was removed by a Pasteur pipette. This step was repeated 4 consecutive times. Then the aqueous solution was transferred to a new clean centrifuge tube, and the hexane extraction procedure was repeated 5 consecutive times. The aqueous solution was transferred into a beaker. The beaker contents were dried in a vacuum desiccator until constant weight was obtained.

3.10 Coating Tenax TA with PFBHA

Tenax TA was cleaned by Soxhlett extraction with methanol at least 24 hours, and then with hexane at least 24 hours. Then, it was dried in a vacuum desiccator until constant weight

was obtained. A weight of 2.0 g of PFBHA was dissolved in 80 mL of distilled water and the solution was added into a 500-mL round bottom flask that contained 18.0 g of the cleaned and dried Tenax TA. Tenax TA was efficiently coated with PFBHA (10 % w/w) using a rotary evaporator while the water was removed under vacuum at 90 ºC. The coated Tenax TA was placed in a vacuum desiccator containing Drierite until constant weight was obtained. The PFBHA coating of 10 % w/w allowed molar excess of PFBHA at the necessary target concentrations of ketones.

3.11 Development of Dynamic Sampling

A dynamic solid sorbent personal air sampling and analysis method was optimized for acetoin, diacetyl, and 2,3-pentanedione vapors using the 2012 ACGIH TLV-TWA for diacetyl.[97] The concentrations 0, 0.1, 0.5, 1, and 2 times the 10 ppb 2012 ACGIH TLV-TWA for diacetyl were studied for the three ketones.

3.11.1 Preparation of Dynamic Samplers

Pyrex glass wool was cleaned by Soxhlett extraction with methanol at least 24 hours, and then with hexane at least 24 hours. Then, it was dried in a vacuum desiccator until constant weight was obtained. A 200 mg of the coated Tenax TA was packed into a Pyrex tube (5-mm ID, 7-mm OD, and 7-cm length) by vibrator, held in place by a 5-mm thick layer of cleaned Pyrex wool in the bottom of the tubes. Once the distance of the sorbent top to the end of the tube was constant on continued vibration, a top 5-mm Pyrex wool layer was inserted. The tubes were stored in a vacuum desiccator at room temperature until sampling. This procedure prevented exposure to ketones, aldehydes, and water vapor.

3.11.2 Preliminary Study with Higher Target Concentrations

The preliminary study had target acetoin and diacetyl concentrations of 0, 0.1, 0.5, 1.0, 1.5, and 2.0 mg/m^3 (0, 28, 139, 277, 416, and 555 ppb of acetoin and 0, 28, 142, 284, 426, and 568 ppb of diacetyl respectively) as they were investigated before the 2011 ACGIH intended TLV and NIOSH REL criteria draft documents were released. Since diacetyl and 2,3-pentanedione are sterically hindered, this may cause low recoveries.[141] Thus, hexane desorption procedures[135, 138, 142] were first tested to see whether acetoin and diacetyl were analyzable without modifying the published methods.

Before testing vapor sampling, ketone liquid spiking was performed. 1 μL of diacetyl and acetoin water solution (0, 1, 2.5, 5, 7.5, and 10 μg/μL) was spiked into an aqueous solution containing PFBHA (10 mg/mL). The concentrations were equivalent to vapor concentrations of 0, 0.1, 0.5, 1.0, 1.5, and 2.0 mg/m^3 when samples were collected for 8 hours at 10 mL/min. Then, the derivatives were extracted with hexane 3 times (1, 0.5, and 0.5 mL), and 2 μL of the solution with M-heptanal internal standard was injected into the GC-MS to find the recoveries. This injection method was utilized for the subsequent procedures in the preliminary study.

1 μL of diacetyl and acetoin in water solvent (0, 1, 2.5, 5, 7.5, and 10 μg/μL) was also spiked onto 200 mg of coated solid sorbent tubes. Then, the tubes were connected to a personal sampling pump and dry air was drawn at 10 mL/min for 1 hour. The derivatives spiked onto the coated solid sorbent were extracted with 2 mL hexane. The hexane solution was manually shaken and allowed ultrasonication for 5 min.

Five different concentrations (0, 0.1, 0.5, 1.0, 1.5, and 2.0 mg/m^3) of acetoin and diacetyl were generated in 10-L Tedlar gas bags by injection of the appropriate mass of ketones in water using the appropriate air volume. Each gas bag in triplicate at each concentration was sampled

with coated Tenax TA tubes connected in series (200 mg front/50 mg back) using a personal sampling pump set at calibrated flow rates of 10 or 50 mL/min. The solid sorbent was suspended in hexane and analyzed using the same procedure as ketone liquid spiking onto the solid sorbent tubes. Also, 200 mg of uncoated Tenax TA was used to sample ketone vapors, and the solid sorbent was suspended in a 2 mL hexane solution containing 20 mg PFBHA.

Since the vapor samples had lower recovery than expected for diacetyl, different desorption procedures were attempted. First, different desorption durations were tested. After acetoin and diacetyl vapors were sampled at 16.0 mg/m^3 for 1 hour (equivalent to 2.0 mg/m^3 8-hour TWA) using the 200 mg coated and uncoated Tenax TA, the solid sorbent was suspended in hexane and analyzed using the same procedure as ketone liquid spiking onto the solid sorbent tubes except the hexane solution was kept at room temperature (25 ºC) for 12, 24, 48, and 72 hours before analysis. Furthermore, the hexane solution was placed in a microwave, in an oven at 60 ºC from 1 hour to 72 hours, or in an ultrasonicator from 1 hour to 72 hours.

The preliminary studies suggested that diacetyl vapor did not completely react with PFBHA in the tube or in the hexane solution. Thus, to determine usable desorption solvents, many solvents with different polarities were tested. The solvents tested were pentane, cyclohexane, methanol, ethanol (with 5 % water), acetonitrile (with 5 % water), nitromethane, chloroform, dichloromethane, tetrahydrofuran (also with 5 % water), and 1,4-dioxane. It was important to use solvents where diacetyl and acetoin O-oximes were stable in the solvent and did not react with it. The dissolved O-oximes were kept at 25 ºC in the solvents over 3 days and injected into the GC-MS to check their kinetics. Solvents in which PFBHA dissolves well are advantageous; thus, PFBHA solubility was tested in each solvent. Furthermore, it was determined whether Tenax TA reacted with solvents. Since it was hard to find a simple solvent

that solved all the problems, a water and hexane mixture was also tested to maximize the recovery.

3.11.3 PFBHA O-Oxime Liquid/Solid Spiking

Before determining reaction efficiency of ketones, desorption efficiencies were determined by spiking an equivalent mass of PFBHA O-oxime derivatives on the solid sorbent tubes. Since M-diacetyl and M-2,3-pentanedione would react with PFBHA on the solid sorbent tubes, only M-acetoin, D-diacetyl, and D-2,3-pentanedione were spiked on the tubes. The three O-oximes were dissolved together in hexane, where O-oxime masses in 30 μL hexane solution were equivalent to their ketone concentrations of 0, 1, 5, 10, and 20 ppb when sampled for 8 hours at 100 mL/min. Then, 30 μL of the hexane solutions were spiked onto the solid sorbent tubes, and dry air was drawn through the sorbent bed for 1 hour at 100 mL/min. The samples were analyzed using Tenax-hexane and Tenax-water-hexane standard curves. For each concentration, the interrun mean recoveries were obtained in triplicate.

3.11.4 Ketone Liquid/Solid Spiking

Reaction efficiencies of the three ketones for liquid/solid spiking were determined by spiking equivalent masses of ketones on the solid sorbent tubes. The three ketones were dissolved together in water, where ketone masses in 5 μL water solutions were equivalent to their ketone concentrations of 0, 1, 5, 10, and 20 ppb when sampled for 8 hours at 100 mL/min. Then, 5 μL of the aqueous solutions were spiked onto the solid sorbent tubes, and dry air was drawn through the sorbent bed for 1 hour at 100 mL/min. The samples were desorbed, extracted, and analyzed by GC-MS using Tenax-hexane and Tenax-water-hexane standard curves. For each concentration, the interrun mean recoveries were obtained in triplicate.

3.11.5 Reaction Time Using Ketone Liquid/Liquid Spiking

Reaction efficiencies of the three ketones for liquid/liquid spiking were also determined by spiking equivalent masses of ketones into a centrifuge tube containing 200 mg of coated Tenax TA in 2 mL of water. The three ketones were dissolved in water for ketone masses in 5 μL water solutions that were equivalent to their ketone concentrations of 10 ppb when sampled for 8 hours at 100 mL/min. Then, 5 μL of the aqueous solution was spiked into a centrifuge tube containing 200 mg of coated Tenax TA and 2 mL of water instead of the actual solid sorbent tube to eliminate possible loss due to handling and transferring solid sorbents. These experiments were undertaken with reaction times of 3 days to determine whether longer reaction times would complete reaction of α-diketones (α-diketones become all di-substituted O-oxime instead of both mono- and di-substituted O-oximes) to simplify the analysis. The samples were treated exactly the same as the ketone liquid/solid spiking sample and analyzed by GC-MS using Tenax-water-hexane standard curves. The interrun mean recoveries were obtained in triplicate.

3.11.6 Calibration of Thermometer and Hygrometer

To generate ketone vapors at known temperature and RH, the multi-function thermal anemometer was calibrated to measure air temperature and RH. The environmental chamber was also calibrated to generate a desired air temperature.

An alcohol thermometer (temperature range from -20 to 150 ºC) was used to calibrate the thermometer of the multi-function thermal anemometer and environmental chamber together. An alcohol thermometer and the multi-function thermal anemometer were placed inside of the environmental chamber whose control was manipulated until the alcohol thermometer read exactly at 5, 25, or 40 ºC.

The hygrometer of the multi-function thermal anemometer was also calibrated for RH using dry air and salt solutions. Saturated solutions of Lithium chloride, calcium chloride, magnesium chloride, potassium carbonate, sodium dichromate, sodium chloride, potassium chloride, and potassium nitrate were created in water at 25 ºC in a desiccator to generate a known RH of air. Dry air generated in a Tedlar gas bag was used for 0 % RH. The calibrated multi-function thermal anemometer was used to obtain temperature and RH simultaneously during the experiment.

3.11.7 Test Atmosphere Preparation

Vapor sampling of the ketones was performed using the static method with Tedlar gas bags. The concentrations evaluated in triplicate were approximately 0, 0.1, 0.5, 1, and 2 times the 10 ppb 2012 ACGIH TLV-TWA of diacetyl, and the same concentrations were tested for acetoin and 2,3-pentanedione over an exposure period of eight hours. By injecting a known volume and concentration of ketones in water into a known volume of air in Tedlar gas bags by a syringe, desired concentration and RH was obtained.

All of the Tedlar gas bags were cleaned with dry air. A gas bag was filled with dry air, heated with a hair dryer for 5 min, and completely emptied via a vacuum. This process was performed 3 times.

To sample 48 L of air at 100 mL/min for 8 hours, 60 L of air was generated in 70- or 100-L Tedlar gas bags. To sample 6 L of air at 100 mL/min for 1 hour, 8 L of air was generated in a 10-L Tedlar gas bag. The volume of air in a gas bag was calculated using fill time and the actual mean flow rate. The flow rate of air was obtained before and after addition of air by an M-5 mini-Buck calibrator. For sampling at 5 and 40 ºC, the gas bags were first prepared at 25 ºC and

they were placed in the environmental chamber at 5 or 40 ºC. Equation 3.1 was used to calculate the volume required at 25 ºC. For example, to create 60 L of air at 5 and 40 ºC, 64.3 and 57.1 L of air were generated at 25 ºC respectively.

$$V_2 = V_1(T_2/T_1) \tag{3.1}$$

To generate 0, 1, 5, 10, and 20 ppb of ketones at 25 ºC in 60 L of air (also to generate 0, 8, 40, 80, and 160 ppb of ketones at 25 ºC in 10 L of air), equation 3.2 was used to covert from ppb to μg/m^3.

$$\text{Conc. in } \mu g/m^3 = \frac{(MW)\times(\text{Conc. in ppb})}{24.45} \tag{3.2}$$

Similarly, to generate 0, 1, 5, 10, and 20 ppb of ketones at 5 or 40 ºC in 60 L of air (also to generate 0, 8, 40, 80, and 160 ppb of ketones at 5 or 40 ºC in 10 L of air), equation 3.3 was used to covert from ppb to μg/m^3 assuming the Ideal Gas Law.

$$\text{Conc. in } \mu g/m^3 = \frac{P\times MW\times(\text{Conc. in ppb})}{R\times T} \tag{3.3}$$

where P is pressure in mm Hg; MW is molecular weight in g/mol; R is the ideal gas constant (62.4 L mm Hg T^{-1} mol^{-1}); T is temperature in Kelvin.

The mass of ketones to inject into the gas bag was calculated using equation 3.4 and the mass of ketones sampled in the tubes was calculated using equation 3.5. Table 3-1 summarizes the mass of ketones injected into the gas bags at 10 ppb at different temperature as examples.

$$\text{Mass in } \mu g = (\text{Conc. in } \mu g/m^3)(\text{Volume of air in L})\left(\frac{1\,m^3}{10^3\,L}\right) \tag{3.4}$$

$$\text{Mass in } \mu g = (\text{Conc. in } \mu g/m^3)(\text{Flow rate in mL/min})(\text{Sampling time in min})\left(\frac{1\,m^3}{10^6\,mL}\right) \tag{3.5}$$

Table 3-1: Mass of Ketone Injected into Gas Bags at Different Temperatures

Temperature (°C)	5	25	40
Ketones (ppb)	10	10	10
Acetoin (μg/m^3)	38.6	36.0	34.3
Diacetyl (μg/m^3)	37.7	35.2	33.5
2,3-Pentanedione (μg/m^3)	43.8	40.9	38.9
Acetoin (μg) in 60 L	2.31	2.16	2.06
Diacetyl (μg) in 60 L	2.26	2.11	2.01
2,3-Pentanedione (μg) in 60 L	2.63	2.46	2.34

To generate 5 and 80 % RH at 5, 25, and 40 ºC, an appropriate amount of water was injected into each gas bag using a syringe assuming the Ideal Gas Law. Table 3-2 shows the volume of water injected into the gas bags when 60 L of air was prepared to sample 48 L of air as an example. The partial pressure of water vapor was first calculated based on the target RH and saturated vapor pressure of water at a given temperature as shown in equation 3.6. Then, the amount of water required in moles was calculated using the Ideal Gas Law equation 3.7. Using the molecular weight of water, the mass of water was calculated since mass does not change at any given temperature. Then, the volume of water to be injected into the gas bag was calculated using the density of water at a temperature where gas bags were prepared (25 ºC).

$$\text{Relative humidity (RH)} = \frac{\text{Partial pressure of water vapor}}{\text{Saturated vapor pressure of water}} \tag{3.6}$$

$$n = \frac{PV}{RT} \tag{3.7}$$

where n is water in mol; P is partial pressure in mm Hg; V is volume of gas bag in L; R is the ideal gas constant (62.4 L mm Hg T^{-1} mol^{-1}); T is temperature in Kelvin.

Table 3-2: Volume of Water Generated in Gas Bags

Saturated Vapor Pressure (mm Hg)	6.54	23.8	55.3
Partial Pressure of Water Vapor (mm Hg) at 5 % RH	0.327	1.19	2.77
Volume of Water (μL) at 5 % RH	20	69	153
Partial Pressure of Water Vapor (mm Hg) at 80 % RH	5.23	19.0	44.2
Volume of Water (μL) 80 % RH	326	1108	2452

Ketones of known mass were dissolved in a known volume of water based on RH in an amber vial, and the appropriate volume of aqueous solution was injected into the gas bag. After a known amount of air, ketones, and water were generated in a clean Tedlar gas bag, the gas bag was heated by a hair dryer to ensure volatilization and mixing of ketones and water. The vapor was removed via a vacuum, and again filled with the desired vapor concentration. This process was performed 3 times, and the third gas bag was retained as the test atmosphere. For the 40 ºC experiment, the gas bag was heated by a hairdryer inside of the environmental chamber due to condensation of water at room temperature. The third gas bag was then placed in the environmental chamber at 5, 25, or 40 ºC.

Because acetoin, diacetyl, and 2,3-pentanedione were known to be light sensitive,[17, 23] the door of the environmental chamber was covered with aluminum foil to avoid light exposure during sampling. The blank gas bag including the same RH without ketones was also added to the chamber. The blank was used as a negative control as well as to check the temperature and RH of the gas bag using the calibrated multi-function thermal anemometer.

The vapor in the gas bag was sampled in the environmental chamber at an air volume of 48 L for 8 hours or 6 L for one hour. The 200-mg coated Tenax TA tubes were used to sample vapors in triplicate at 100 mL/min using a calibrated personal pump and Teflon tubing connected by a butt-to-butt joint with Tygon tubing. The flow rate of the pump was calibrated by a DryCal

before and after sampling 3 times each. The temperature of the environmental chamber and atmospheric pressure were obtained three times during sampling.

After sampling, the Pyrex wool was removed from the tubes, and the 200-mg coated Tenax TA was desorbed in water and extracted with hexane as detailed in Section 3.8. Thus, the standard and samples were treated in the same manner. 10 μL of internal standard solution (0.05 mg/mL in hexane) was spiked into 190 μL of the sample hexane extracted aqueous solution. An aliquot (2 μL) was injected into GC-MS, and "Tenax-water-hexane" standard curves were used to find the recovery of ketone vapors sampled in the dynamic sampling tubes. If there were peaks in the blank at the critical retention times, they were subtracted from the sample concentrations. Recoveries were calculated based on the theoretical concentration in the gas bags using actual flow rate of the pump, volume of air generated, and duration of time sampled.

3.11.8 Comparison of Individual Ketone Vapors and Their Vapor Mixture

Acetoin, diacetyl, and 2,3-pentanedione vapors at 80 ppb at 25 ºC at 5 % RH were sampled individually in triplicate for 1 hour at 100 mL/min. The three ketones as a vapor mixture at 80 ppb each at 25 ºC at 5 % RH were sampled in triplicate for 1 hour at 100 mL/min. The recovery for each individual ketone vapor was compared to the three ketones as a vapor mixture. A two-tailed Student t-test was performed assuming unequal variance at $\alpha = 0.05$, and p-values were calculated to determine whether means were statistically different.

3.11.9 Comparison of Vapor Sampling Time

The ketone vapor mixture at 10 ppb at 25 ºC at 5 % RH was sampled in triplicate for 8 hours at 100 mL/min. The ketone vapor mixture at 80 ppb at 25 ºC and 5 % RH was sampled in triplicate for 1 hour at 100 mL/min. Thus, the mass of ketones collected in the tubes were

theoretically the same. The recoveries for 1-hour samples and 8-hour samples were compared for the three ketones.

3.11.10 Comparison of RH during Vapor Sampling

The ketone vapor mixture at 0, 1, 5, 10, and 20 ppb at 25 ºC at 5 or 80 % RH was sampled in triplicate for 8 hours at 100 mL/min. The recovery for 5 % RH samples and 80 % RH samples were compared for the three ketones. Although the PFBHA-coated Tenax TA method was shown from previous studies to have no effects from humidity and temperature for aldehydes, humidity and temperature effects have to be shown directly for acetoin, diacetyl, and 2,3-pentanedione.

3.11.11 Comparison of Temperature during Vapor Sampling

The ketone vapor mixture at 20 ppb at 5 and 80 % RH at 5, 25, or 40 ºC was sampled in triplicate for 8 hours at 100 mL/min. The recoveries for 5, 25, and 40 ºC samples were compared for the three ketones.

3.11.12 Sampling Capacity

Dynamic personal air sampling through two tubes connected in series (200/200 mg) was used to test the capacity of the tube or breakthrough of the front tube. Although this method was developed for low ppb level sampling for 8 hours, it would be useful if this sampling method could be used at higher concentration range. Thus, air sampling was conducted using the three ketones separately and as vapor mixtures at 5 % RH at 100 mg/mL for 1 hour. For vapor mixtures, the same concentration range was tested at 5 and 80 % RH. For 5 % RH, methanol was used to dilute the ketone. The recoveries were corrected using the desorption and reaction

efficiencies of each ketone at the concentration for which no breakthrough occurred. Sampling capacity was determined where the front tube recovery met the NIOSH criterion of > 75 % recovery.[148] At high concentrations, the samples were diluted into the working linear range before GC-MS quantitation with the Tenax-water-hexane method blank. Then the capacities of the sampler were determined in terms of the ketone number of moles.

3.11.13 Determination of Dynamic Sampling Tube Storage Periods after Sampling

In a real situation, such as when an industrial hygienist collects air samples and sends them to a lab, it is hard to analyze the samples the same day they are collected. Thus, it is important to find the storage periods of the solid sorbent tubes at room temperature and when a refrigerator or freezer storage is available. Therefore, to assess whether sample storage at different temperatures affected recoveries, temperature and duration of storage were compared.

Acetoin, diacetyl, and 2,3-pentanedione vapors as a mixture were sampled at 80 ppb at 100 mL/min for 1 hour (equivalent to 10 ppb 8-hour TWA) at 25 ºC at 5 and 80 % RH. After the sampling, each tube was wrapped with aluminum foil. Then, the tubes were stored in a freezer (-20 ºC), a refrigerator (5 ºC), or at room temperature (25 ºC). The recoveries for the ketones were calculated when samples were stored for 0, 3, and 30 days after sampling. For different combinations of storage temperatures and durations, triplicate samples with one blank were analyzed using Tenax-water-hexane standard curves.

3.12 Development of Passive Sampling

After dynamic air sampling and analysis for acetoin, diacetyl, and 2,3-pentanedione was optimized, a passive sampling method was also established because passive sampling has many advantages for workers. Unlike dynamic sampling, passive samplers do not require pumps.

Thus workers can move freely even if they are wearing several passive samplers. A new solid sorbent passive sampler was developed using a 10 % (w/w) PFBHA coated Tenax TA pellet in order to sample acetoin, diacetyl, and 2,3-pentanedione at the ppb level.

3.12.1 Preparation of Mini-Passive Samplers

As a preliminary study, a modified "mini-pellet" was prepared first according to the method published by Tsai et al.[137, 139-141] 200 ± 2 mg of the coated Tenax TA was used to produce a 13-mm diameter and 3-mm thick pellet by a Parr 2811 bench manual pellet press that developed 0.5 ton force. The pellet was placed in a Teflon-lined screw cap of dimensions 14-mm ID, 18-mm OD, 14-mm internal depth, and 16-mm outer height. Since the thickness of the pellet was 3.0 ± 0.1 mm, the diffusion path length was 11 mm. Figure 3-1 (not to scale) demonstrates the schematic of the "mini-passive sampler."

The 3M model 3500 organic vapor monitor[149, 150] was disassembled to use parts of the sampler. The Teflon stay of the monitor was cut and placed on the pellet to secure the pellet and allow a constant diffusion path. The silicone membrane was placed over the screw cap and secured tightly with an aluminum seal via a crimper. For field sampling, the screw cap was secured centrally to the bottom of the empty 3M outer casing by a small piece of duct tape. The samplers were stored in a vacuum desiccator at room temperature until sampling to prevent exposure to ketones, aldehydes, and water vapor.

For the mini-passive samplers, the theoretical sampling constant k was calculated using equation 2.8 in Section 2.8. Since A/L was $(1.3/2)^2 \times \pi \times 1/1.1 = 1.2$ cm, the theoretical sampling constant k at 25 ºC for acetoin was $k = D_{AB}A/L = 0.087 \times 1.2 = 0.11$ cm^3/sec = 6.3 mL/min. Similar calculation determined the sampling constant k at 25 ºC for diacetyl and 2,3-

pentanedione as 6.4 and 5.8 mL/min respectively. The mini-passive samplers have been used to sample aldehydes and non-sterically hindered ketones.[137, 139-141]

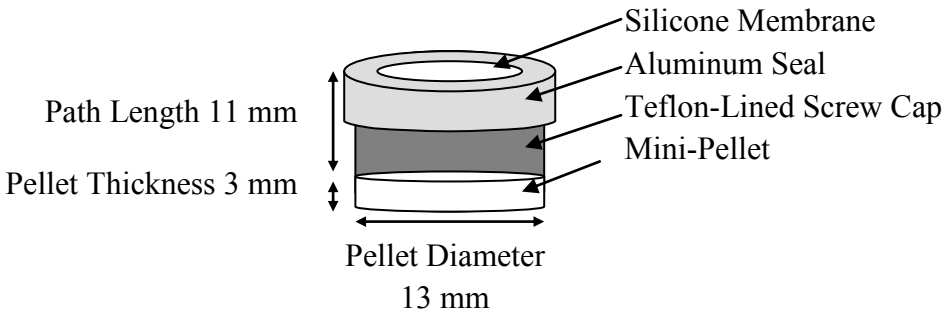


Figure 3-1: Schematic of Mini-Passive Sampler

3.12.2 Development of Custom Passive Samplers

Because the mini-passive sampler was not sensitive enough to measure acetoin, diacetyl, and 2,3-pentanedione at the low ppb level, a custom passive sampler was developed with the required sensitivity. In order to increase the sensitivity, the sampling constant had to be increased by increasing the pellet diameter and decreasing the path length as shown in equation 2.8 from Section 2.8.

Because the sampler must be a practical size for workers to wear, the sampler could not be oversized. 3M provided a sampler that was large enough to hold a pellet of practical size. The sampler already came with a silicone membrane, so it was easier to adapt into a custom sampler. Thus, the diameter of the pellet (30 mm) was chosen based on the ID of the sampler, and a pellet press with the desired 30-mm ID die set was utilized. Also, the minimum mass of the solid sorbent and pressure of the pellet press was identified to ensure pellets could be constructed without cracking or breaking and to retain the microporous characteristic of Tenax TA.

Since a 100 mL/min flow rate provided enough sensitivity to sample at least 0.5 times the 2012 ACGIH TLV-TWA for diacetyl, the theoretical sampling constant needed to be at least 100 mL/min. Thus, the desired path length was identified by equation 2.8 using the given diameter (30 mm) of the pellet and the desired flow rate (100 mL/min). From the calculation, the path length must be less than 3.7, 3.8, and 3.4 mm for acetoin, diacetyl, and 2,3-pentanedione respectively at 25 ºC. However, the 3M holder had a height greater than the desired path length, so the height was adjusted by finding acrylic discs to heighten the pellet.

1-1/4 in (32 mm) diameter clear acrylic circles were resized to 30-mm diameter to fit within the 3M sampler. To reduce the outside diameter of the acrylic discs from 32 mm to 30 mm, a piece of aluminum round stock was machined to a diameter of 32 mm by securing to the chuck of a lathe. This process was performed by UCLA Engineering Research and Development Shops. 300 mg of the coated Tenax TA was used to produce a 30-mm diameter and 0.53 mm thick pellet by a MP15 laboratory pellet press with built-in hydraulic pump and a 30-mm ID dry press die set. The coated Tenax TA was evenly spread in the die set and was pressed with 6 tons of pressure. The pellet was placed in the empty 3M outer casing on top of two 30-mm clear acrylic circles, producing a diffusion path length of 3.2 mm. The silicone membrane was placed over the 3M sampler, and the plastic ring was snapped on. Figure 3-2 (not to scale) demonstrates the schematic of the "passive sampler." The samplers were stored in a vacuum desiccator at room temperature until sampling to prevent exposure to ketones, aldehydes, and water vapor.

To ensure that the pellet weight, pellet thickness, and diffusion path length were precisely produced, the mean, SD, and CV were obtained among 90 samplers. The diffusion path length was calculated by subtracting the thickness of the silicone membrane, pellet, acrylic discs, and

bottom of the sampler from the height of the entire sampler. The thickness and height of each individual component was measured by an electronic digital micrometer.

For the custom developed passive samplers, the theoretical sampling constant k was calculated using equation 2.8 in Section 2.8. Since A/L is $(3/2)^2 \times \pi \times 1/0.32 = 22$ cm, the theoretical sampling constant k at 25 ºC for acetoin was $k = D_{AB}A/L = 0.087 \times 22 = 1.9$ cm^3/sec = 114 mL/min. Similar calculation determined the sampling constant k at 25 ºC for diacetyl and 2,3-pentanedione as 117 and 105 mL/min respectively. Similar calculation determined the sampling constant k at 40 ºC for acetoin, diacetyl, and 2,3-pentanedione as 124, 127, and 115 mL/min respectively.

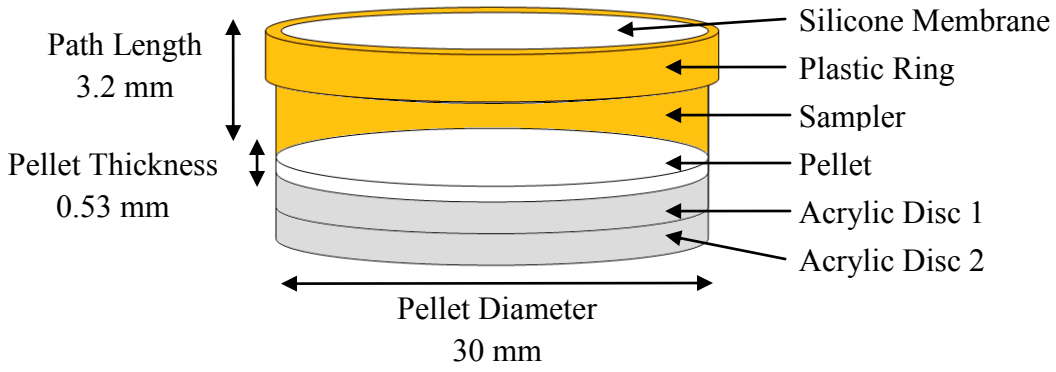


Figure 3-2: Schematic of Custom Developed Passive Sampler

3.12.3 Calibration of Anemometer and Syringe Pump

The multi-function thermal anemometer was calibrated to measure air velocity. Since air flow rate (Q) is equal to the air velocity (V) times the cross-sectional area (A), air velocity was calibrated using an air stream through a tubing of known area. The anemometer probe was placed in front of the 1/4-in ID tube end while air was generated. Before and after the air velocity was measured, the air flow rate was measured in triplicate by an M-5 mini-Buck

calibrator. Using the measured air flow rate, expected air velocity was calculated, which was compared to the observed air velocity.

The syringe pump was also calibrated using water. Water at 25 ºC was drawn into a gas tight syringe, and the syringe was set on the syringe pump at different flow rates for one hour. The water loss was measured using an analytical balance by checking the weight of syringe before and after the water loss. The volume loss was calculated using the density of water at 25 ºC. A Hamilton 5-mL gas tight syringe was used to calibrate flow rate of 0.1, 0.3, 0.5, 0.7, and 1.0 mL/hr and a Hamilton 50-mL gas tight syringe was used to calibrate flow rates of 2, 3.5, 5, 6.5, and 8 mL/hr.

3.12.4 PFBHA O-Oxime Liquid/Solid Spiking

Desorption efficiencies of O-oximes were determined by spiking an equivalent mass of O-oxime on the coated Tenax TA pellets in the 3M sampler. Since M-diacetyl and M-2,3-pentanedione would react with PFBHA on the solid sorbent pellets, only M-acetoin, D-diacetyl, and D-2,3-pentanedione were spiked onto the pellets. The three O-oximes were dissolved together in hexane. O-oxime masses in 30 μL hexane solution were equivalent to their ketone concentrations of 0, 1, 5, 10, and 20 ppb when sampled for 8 hours at the theoretical sampling constants at 25 ºC given in Section 3.12.2. Then, 30 μL of the hexane solutions were spiked onto the pellet slowly in circles from the outer side of the pellets towards the center. The spiked pellets were placed in a desiccator overnight at 25 ºC to dry the hexane before desorption.

The pellet was transferred onto weighing paper, and a spatula tip was used to divide the pellet into three strips. The pellet pieces were transferred into a 15-mL centrifuge tube, and the pieces were mashed into powder form by the spatula. A volume of 2 mL of water was added

into the tube containing 300 mg of the PFBHA coated Tenax TA. The tube was manually shaken vigorously for 1 min, placed in an ultrasonicator for 2 min, heated in a microwave oven until the first bubbles appeared in the aqueous solution, and set at room temperature for 1 hour. 2 mL of hexane was added into the aqueous solution and the water/hexane solution was shaken for 1 min and centrifuged for 20 min. 10 µL of the M-heptanal internal standard (50 µg/mL) was added into 190 µL of the hexane solution, and the solution was shaken manually. An aliquot (2 µL) was then injected into the GC-MS for internal standard analysis. The "Tenax-water-hexane" standard curves were utilized using 300 mg of the PFBHA coated Tenax TA instead of 200 mg as described in Section 3.8.3. For each concentration, the interrun mean recoveries were obtained in triplicate.

3.12.5 Ketone Liquid/Solid Spiking

Reaction efficiencies of the three ketones for liquid/solid spiking were determined by spiking ketones onto the solid sorbent pellets in 5 µL of methanol solution. The three ketones were dissolved together in methanol, where ketone masses in 5 µL were equivalent to their ketone concentrations of 0, 1, 5, 10, 20, 30, and 40 ppb when sampled for 8 hours at the theoretical sampling constants at 25 ºC given in Section 3.12.2. Then, 5 µL of the solution was spiked onto each pellet slowly in circles from the outer side of the pellets towards the center. Each spiked pellet was placed in a glass jar covered with aluminum foil overnight at 25 ºC to dry the methanol before desorption. The pellet was desorbed and analyzed as described in Section 3.12.4.

3.12.6 Test Atmosphere Preparation

A pure dry compressed air cylinder, calibrated rotameter, syringe pump, mixing chamber, exposure chamber, and interconnecting tubing were connected as shown in Figure 3-3. The compressed air cylinder was connected to a rotameter by Tygon tubing, and the air flow range used was 1000-5000 mL/min. The flow rate of air was calibrated before and after generation by an M-5 mini-Buck calibrator. The Tygon tubing was connected to Teflon tubing, which was connected to stainless steel tubing. Acetoin, diacetyl, and 2,3-pentanedione in water equivalent to 0, 5, 10, 20, 30, and 40 ppb 8-hour TWA at 5 and 80 % RH were prepared and drawn into a gas tight syringe. In the preliminary study, higher concentrations at 0, 0.1, 0.5, 1.0, 1.5, and 2.0 mg/m^3 8-hour TWA (0, 28, 139, 277, 416, and 555 ppb of acetoin and 0, 28, 142, 284, 426, and 568 ppb of diacetyl respectively) were also tested before the 2011 ACGIH intended TLV-TWA and NIOSH REL criteria draft documents were released.

The syringe was placed onto a calibrated syringe pump, which was set at the desired velocity to generate the target concentration of ketone and water vapors. The needle of the syringe was inserted into a stainless steel swagelok injection port containing Thermogreen septa. The stainless steel tubing and swagelok were wrapped by heating tape to ensure complete volatilization of ketones and water. The heating tape was heated using a variable transformer, and the temperature between the stainless steel tubing and heating tape was 120 ºC. The stainless steel tubing wrapped by heating tape was connected to Teflon tubing, which was then connected to a 25-mL midget impinger mixing chamber by butt-to-butt joints with Tygon collars. It was connected by Teflon tubing to a hole drilled in the side near the bottom of a Bel-Art vacuum desiccator acting as an exposure chamber. The end of the Teflon tubing was set in the center on the bottom of the chamber, and a fan was set above the tubing to ensure equal

distribution of ketone and water vapors. The fan was also used to maintain a constant face velocity, which exceeded the critical face velocity of 15-20 ft/min.[140] The 23-cm diameter ceramic metal plate containing 11-mm diameter holes and a central 30-mm hole was placed above the fan. The passive samplers were set on the ceramic metal plate.

In the side of the chamber, just above the passive samplers, two holes were bored to allow insertion of the Kanomax multi-function thermal anemometer for continuous measurement of air temperature, RH, and air velocity during sampling. Also, a Kontes 3-place gas sampling manifold was used to connect three dynamic sampling tubes to one hole of the exposure chamber. The dynamic sampling tubes were wrapped with aluminum foil to eliminate light exposure during sampling. Dynamic sampling tubes were inserted for simultaneous monitoring of ketone vapor concentration. The detailed dynamic sampling procedures are provided in Section 3.11. The far end of the manifold was connected to a pump at 400 mL/min to provide a positive head pressure for sampling. The whole system except the compressed air cylinder was set in a chemical fume hood to minimize the ketone exposure, and Teflon tubing vented excess vapor to the same fume hood. A silicone half mask was also required to perform this experiment to minimize the ketone exposure. The Snoop liquid leak detector was used to check the whole system for air leaks. The solid sorbents of the passive samplers were desorbed and analyzed using the optimized method as mentioned in 3.12.4 The solid sorbent of the dynamic samplers were desorbed and analyzed using the optimized method as mentioned in Section 3.8.3 except that 100 mg of coated Tenax was added to generate a total of 300 mg. This allowed common calibration curves for both dynamic and passive samples.

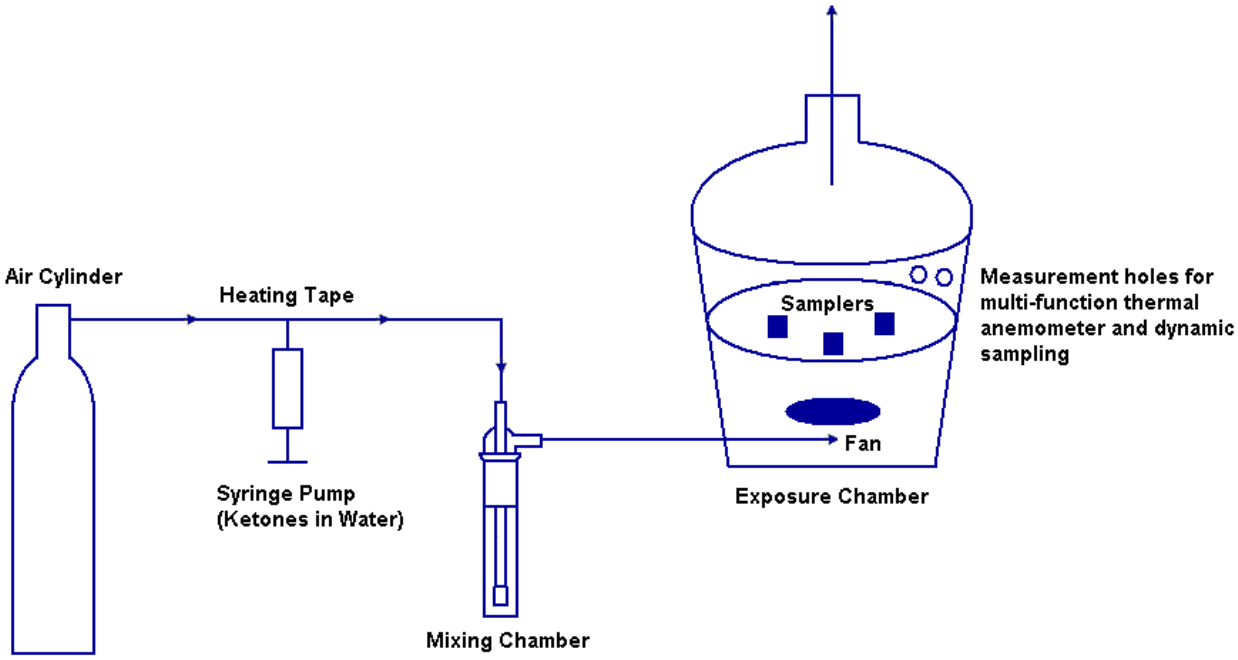


Figure 3-3: Test Atmosphere Generation and Sampling Apparatus

3.12.7 RH and Concentration in Exposure Chamber

Since air, ketones, and water vapor traveled through the tubing and filled the exposure chamber, it took time for the chamber to reach the target ketone concentration and RH. In order to understand the appropriate time to start sampling, the RH was continuously monitored every minute over time at 25 ºC.

First, dry air without water injection was monitored to identify the time for the RH in the chamber to stabilize. The lid of chamber was opened and the ambient RH and temperature were obtained. Once air flow at 3000 mL/min was generated, the RH and temperature inside of the chamber was continuously monitored every minute using a multi-function thermal anemometer. Similarly, the target RH of 5 and 80 % was also monitored when the air flow and syringe pump were simultaneously started.

Furthermore, when passive samplers were inserted into the chamber, the condition of the exposure chamber changed. Since it took 10 seconds to place the samplers and required the lid to be opened 3 inches, the effect of opening the lid was also assessed. Since it was inappropriate to perform continuous direct measurements of ketone concentrations at the ppb level by PID, RH was continuously monitored during ketone exposure to ensure the environment integrity assuming complete volatilization of ketones.

Also, it was assessed whether adsorption of ketones occurred on the Teflon and stainless steel tubing and the inner walls of the mixing chamber. The amount adsorbed was determined by rinsing the Teflon tubing and mixing chamber individually with 2 mL of distilled water. Each 2 mL aqueous solution was transfer into a 15-mL centrifuge tube, and 20 mg of PFBHA was added to each tube. Then, the solution was microwaved for 10 seconds, cooled for 1 hour, and extracted with 2 mL of hexane. An aliquot (2 μL) of hexane solution was injected into the GC-MS to identify possible existence of PFBHA O-oxime peaks from acetoin, diacetyl, and 2,3-pentanedione.

The optimized dynamic sampling method was utilized to identify the actual ketone concentration in the chamber. The actual concentration was determined by identifying the mass of PFBHA O-oximes detected in the GC-MS, extrapolation to the desorption volume, the equivalent ketone mass, the average pump flow rate for each pump, the sampling time, and the air sampling volume. Then, the sampling efficiency for each ketone was also used to obtain the actual concentration of the ketone vapor. The acetoin, diacetyl, and 2,3-pentanedione vapor dynamic sampling efficiencies were 90.2 ± 6.9, 92.2 ± 5.9, and 82.5 ± 4.4 % respectively when the vapors were sampled at 5, 10, and 20 ppb; 25 °C; both 5 and 80 % RH; and 100 mL/min for 8 hours as described in Section 4.7.8.

3.12.8 Determination of Experimental Sampling Constants

The experimental sampling constants of acetoin, diacetyl, and 2,3-pentaneidone relative to the pellet were determined using the vapor generation and exposure chamber system in Figure 3-3. After the total mass of ketone derivatives sampled in pellets was determined by GC-MS analysis, the total mass on the pellet was calculated for each ketone. Then, the total mass of equivalent ketone sampled over sampling time (μg) (Y axis) was plotted against the equivalent ketone air true concentration corrected by dynamic sampling inefficiency (μg/mL) × sampling time (min) (X axis). From the linear plot, the slope of the linear regression line was obtained, and the slope was the experimental sampling constant (mL/min). The theoretical sampling constants were calculated at each temperature using the experimental path length for comparison as mentioned in Section 3.12.2.

Furthermore, whether there was any significant difference between the intercept of experimental sampling constant and zero was determined with via a two-tailed Student t-test. To compare the two means (intercept and zero), the SD and observation number (n) were assumed to be equal. The degree of freedom was calculated as $n_1 + n_2 - 2$, and a significant level of $\alpha = 0.05$ was used. The t-value was calculated using equation 3.8

$$\text{Student T test: } T = \frac{x_1 - x_2}{\sqrt{\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_2}}} \tag{3.8}$$

The critical T value was obtained from a t-distribution table.[151] When the calculated t-value was smaller than the critical value, the intercept was statistically the same as zero. When the calculated t-value was larger than the critical value, the intercept was statistically different

from zero at $p \leq 0.05$. Equation 3.8 was also used to compare experimental sampling constants by using a slope as the mean and a SD of the slope as the SD.

3.12.9 Sampling Temperature, RH, and Duration Effects

To determine whether sampling temperature, RH, and duration affected the sampling constants for acetoin, diacetyl, and 2,3-pentanedione, 0, 5, 10, 20, 30, and 40 ppb 8-hour TWA equivalents were generated in the exposure chamber at 5 and 80 % RH at 25 and 40 ºC for 1 or 8 hours. The amount of ketones and water required to generate the vapors were calculated as shown in Section 3.11.7, and the syringe pump and air flow rate were set to produce the desired concentration of ketones and RH. The lower quantifiable limits for the passive air sampling method were determined where the CV was less than 10 %.

The experiment was performed as described in Section 3.12.6 at 25 ºC, however, testing at 40 ºC required a slight change to the setup. The exposure chamber was placed in a Thermolyne Series 5000 Incubator. As the incubator door remained open to accommodate the experimental apparatus, the incubator temperature was set high enough to maintain 40 ºC within the exposure chamber. The heating tape was also wrapped around the mixing chamber and the Teflon tubing between the mixing and exposure chambers. The inner chamber temperature, RH, and the face velocity of the sampler were continuously measured with the calibrated multi-function thermal anemometer.

3.12.10 Sampling Capacity

The capacity of the pellet was determined by testing various high vapor concentrations. Air sampling was conducted using the three ketones separately and as vapor mixtures at 80 % RH at 25 ºC for 1 to 8 hours. The concentration generated in the exposure chamber was

confirmed via the optimized PFBHA coated Tenax TA dynamic sampling tubes. Depending on the concentration, the pump flow rates ranged from 5 to 100 mL/min to minimize the breakthrough. Font and back tubes were used in series to determine whether breakthrough existed. Because of high concentrations, the samples were diluted into the working linear range before GC-MS quantitation by method blank hexane solution (Tenax-water-hexane). Then, moles of ketones collected on the pellet (μmol) were plotted against the air concentration (μmol/mL) × sampling time (min), and the saturation points were identified by assessing the asymptote in O-oxime mass desorbed.

3.12.11 Determination of Passive Sampler Storage Periods after Sampling

Acetoin, diacetyl, and 2,3-pentanedione vapors as a mixture were sampled at 80 ppb for 1 hour (equivalent to 10 ppb 8-hour TWA) at 25 ºC and 5 % RH. After the sampling, the plastic ring and silicone membrane were removed. The closure cap was snapped firmly onto the sampler and the two port plugs were also firmly seated. The sampler was placed into the 3M can and sealed with the plastic lid. The samplers were stored in a freezer (-20 ºC) or at room temperature (25 ºC). The recoveries for the ketones were calculated when samples were stored for 0, 3, and 30 days after sampling. For different combinations of storage temperatures and durations, triplicate samples with one blank were analyzed using Tenax-water-hexane standard curves.

4 Results

The results from the development of dynamic and passive air sampling methods for acetoin, diacetyl, and 2,3-pentanedione are provided. Some of the raw data are provided in the Appendix.

4.1 Synthesis of PFBHA O-Oximes

Table 4-1 shows the results of PFBHA O-oxime synthesis in terms of GC-MS purities and reaction yields in percent for triplicate synthesis. All yields and GC-MS purities for mono-substituted O-oximes exceeded 90 %. Ketones turned into a mono-substituted O-oxime in aqueous solution in one hour at room temperature after microwaving when about one mole of PFBHA was present.

D-diacetyl with one hour reaction time had lower PFBHA O-oxime yields and purities because it contained M-diacetyl as well. When mono- and di- substituted derivatives were summed, they were actually 99 % of the original diacetyl. The yield and GC-MS purity of D-diacetyl increased from 73.8 ± 2.8 and 88.8 ± 8.3 % respectively at 1-hour to 88.7 ± 3.8 and 99.3 ± 0.7 % respectively after 2 days. The yield and GC-MS purity of D-2,3-pentanedione increased from 80.5 ± 1.4 and 80.8 ± 5.5 % respectively at 2 days to 86.8 ± 1.0 and 96.0 ± 0.3 % respectively after 5 days. Even after 5 days of synthesis, un-reacted M-2,3-pentanedione remained to the extent of 3.0 ± 0.2 %.

Table 4-1: Yield and GC-MS Purities of PFBHA O-Oximes

PFBHA O-Oxime	PFBHA:Ketone	Reaction Time	Raw Yield (%)	GC-MS Purity (%)
M-Heptanal	1:1	1 hour	96.40 ± 0.36	99.755 ± 0.039
M-Acetoin	1:1	1 hour	98.5 ± 3.9	95.00 ± 0.79
M-Diacetyl	1:1.2	1 hour	94.90 ± 0.89	95.18 ± 0.37
M-2,3-Pentanedione	1:1.2	1 hour	97.97 ± 0.51	96.73 ± 0.11
D-Diacetyl	2.3:1	1 hour	73.8 ± 2.8	88.8 ± 8.3
D-Diacetyl	2.3:1	2 days	89.7 ± 3.8	99.30 ± 0.74
D-2,3-Pentanedione	2.3:1	2 days	80.5 ± 1.4	80.8 ± 5.5
D-2,3-Pentanedione	2.5:1	5 days	86.77 ± 0.96	95.95 ± 0.25

4.2 GC Temperature Program Optimization

GC-MS TIC analysis showed that E- and Z-isomers of the PFBHA O-oximes had the same molecular ion cluster and fragmentation pattern with a dominant m/z 181 base peak of the 2,3,4,5,6-pentafluorotropylium ion. M-2,3-pentanedione and D-2,3-pentanedione had different constitutional (structural) isomers. Table 4-2 summarizes the retention times of the E- and Z-isomers of the O-oximes as well as impurities, such as un-reacted PFBHA and formaldehyde O-oximes with TIC analysis. Table 4-2 also provides the key m/z used to identify each O-oxime. Table 4-3 summarizes the retention time of E- and Z-isomers of O-oximes with SIM analysis. The retention time shifted slightly upon each injection, but the relative retention time between each PFBHA O-oxime peak and the internal standard remained constant.

Figure 4-1 and Figure 4-2 provide the gas chromatograms when the method blank solution was injected into the GC-MS and monitored with SIM m/z 181 and 240 or SIM m/z 240 respectively. SIM with m/z 240 had fewer background peaks compared to SIM with both m/z 181 and 240. Figure 4-3 and Figure 4-4 provide the gas chromatogram when the sample solution of acetoin, diacetyl, and 2,3-pentanedione vapors in mixture were injected into the GC-MS and

monitored with SIM m/z 181 and 240 or SIM m/z 240 respectively. SIM with m/z 240 presents the M-acetoin peak without M-2,3-pentanedione peaks.

Table 4-2: Retention Times in the TIC Optimized Temperature Program for Purity

Chemical	PFBHA O-Oxime	Retention Time (min)	m/z
Formaldehyde	Formaldehyde O-oxime	6.50	181; 195
PFBHA		7.21	161; 181
Diacetyl	M-Diacetyl	9.06; 9.25	181; 281
	D-Diacetyl	12.17; 12.53; 13.05	181; 476
Acetoin	M-Acetoin	9.71 (no separation)*	181; 240
2,3-Pentanedione	M-2,3-Pentanedione	9.53; 9.58; 9.77	181; 295
	D-2,3-Pentanedione	12.45; 12.71; 12.74; 13.12	181; 490
n-Heptanal	M-Heptanal	10.60 (no separation)*	181; 239; 309

* When a longer temperature program was used, there was separation of the E- and Z- isomer peaks, but this short optimized program showed only one peak because their isomers had close retention times.

Table 4-3: Retention Times in the SIM Optimized Temperature Program for Quantitation

Chemical	PFBHA O-Oxime	Retention Time (min)
Diacetyl	M-Diacetyl	4.88; 5.13
	D-Diacetyl	10.71; 11.42; 12.35
Acetoin	M-Acetoin	5.96 (no separation)*
2,3-Pentanedione	M-2,3-Pentanedione	5.68; 5.75; 6.06
	D-2,3-Pentanedione	11.23; 11.76; 11.80; 12.51
n-Heptanal	M-Heptanal	7.59 (no separation)*

* When a longer temperature program was used, there was separation of the E- and Z- isomer peaks, but this short optimized program showed only one peak because their isomers had close retention times.

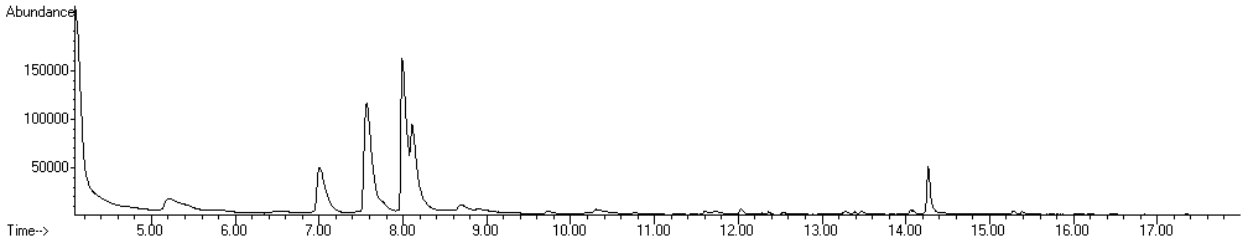


Figure 4-1: Gas Chromatogram of Blank Using SIM m/z 181 and 240

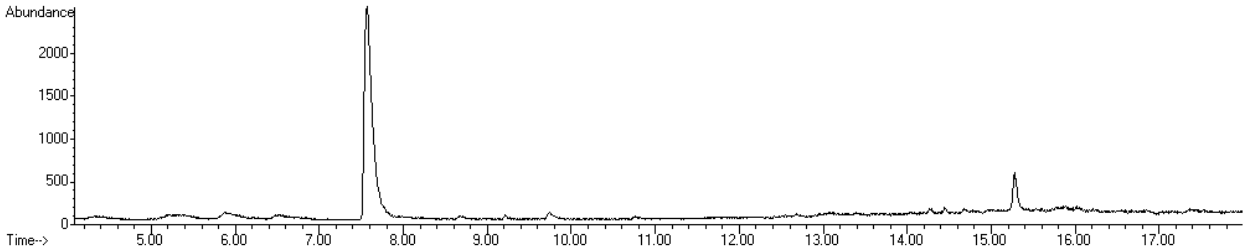


Figure 4-2: Gas Chromatogram of Blank Using SIM m/z 240

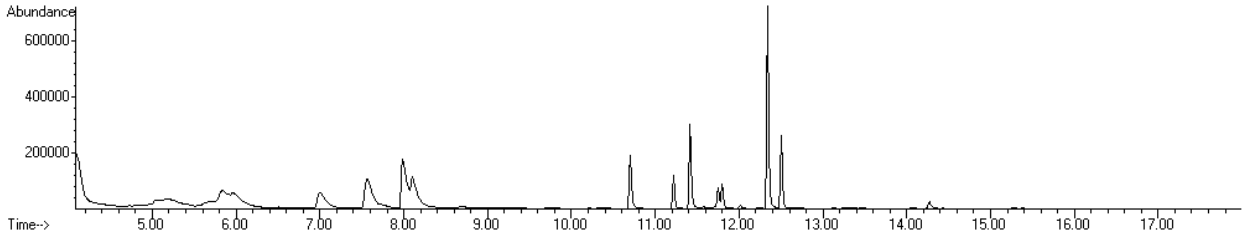


Figure 4-3: Gas Chromatogram of the Vapor Mixture Sample Using SIM m/z 181 and 240

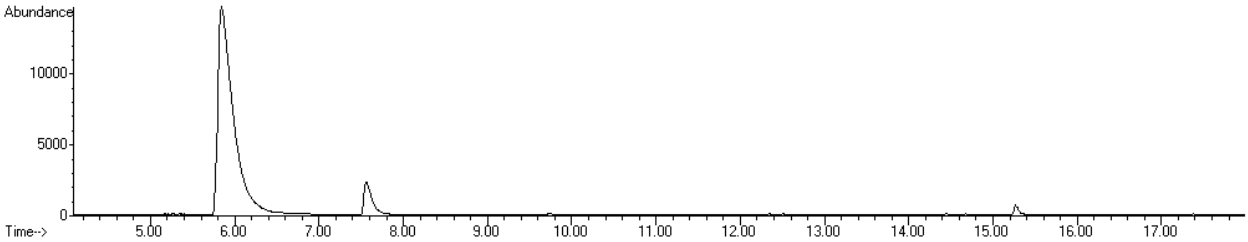


Figure 4-4: Gas Chromatogram of the Mixture Sample Using SIM m/z 240

4.3 Selection of Internal Standard

Figure 4-5 shows the AUC of the 4 injected chemicals over time and Figure 4-6 shows the AUC ratios of target O-oxime to internal standard. Figure 4-5 shows that the area of M-diacetyl and D-diacetyl closely followed the change in the area of the M-heptanal internal standard. Figure 4-6 shows that the AUC ratio remained stable over 8.5 hours using M-heptanal as an internal standard. In fact, the mean AUC ratio of M-diacetyl/M-heptanal over 8.5 hours was 0.806 ± 0.050 with less than 10 % CV of 6.3 %. Similarly, the mean AUC ratio of D-diacetyl/M-heptanal over 8.5 hours was 1.069 ± 0.042 with a CV of 3.9 %. Thus, the results

showed that M-heptanal was a better internal standard compared to DBP in this analysis and was used in the optimized method.

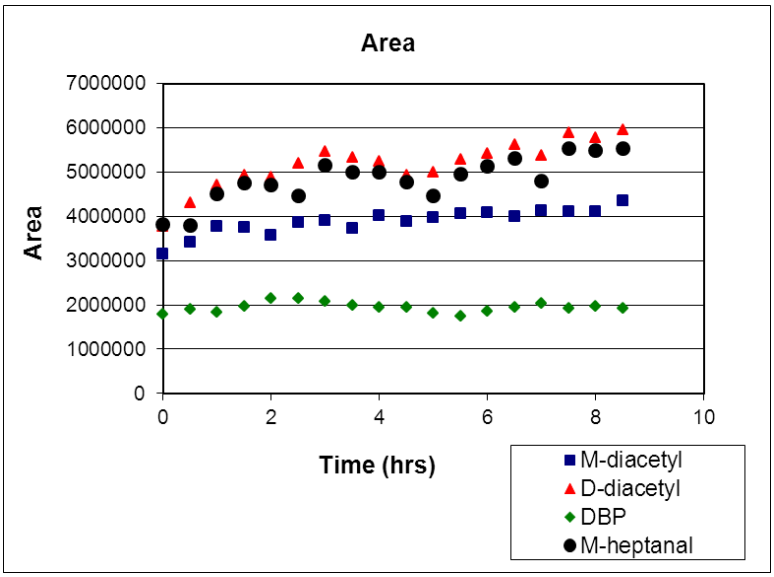


Figure 4-5: AUC vs Time for M-Diacetyl, D-Diacetyl, DBP, and M-Heptanal

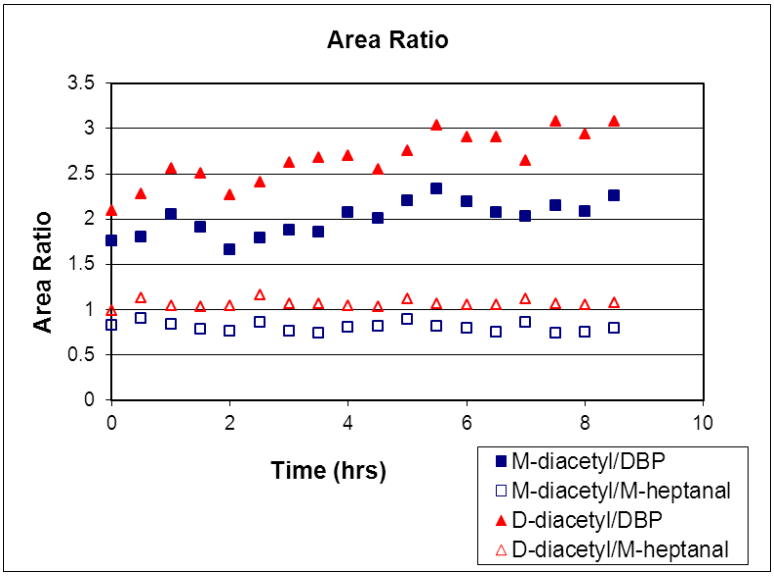


Figure 4-6: AUC Ratio vs Time for Target PFBHA O-Oxime/Internal Standard

4.4 E- and Z- Isomer Ratios of PFBHA O-Oximes

To verify whether E- and Z- isomer ratios remained constant at different tested conditions, triplicate samples were analyzed, and the interrun means, SDs, and CVs were compared as shown in Table 7-1 in the Appendix for D-diacetyl.

Table 4-4 indicates p-values to compare the means of isomer ratios of D-diacetyl which was the largest peak. P-values of less than 0.05 indicate that the largest D-diacetyl isomer ratio was significantly different. Most importantly, the isomer ratios of Tenax-Water-Hexane standard and the ketone vapor/solid spiking were statistically different at $p \leq 0.05$. Thus, all of the isomers needed to be combined for quantification as they had different ratios at different conditions compared to actual air sampling (vapor spiking). The sum of all isomers was used for further analysis for all PFBHA O-oximes of acetoin, diacetyl, and 2,3-pentanedione.

Furthermore, there were E- and Z- isomers of D-diacetyl and D-2,3-pentanedione when diacetyl and 2,3-pentanedione reacted with PFBHA in 95:5 ethanol:water solution and were injected into a GC-MS. Although the GC column used in this research was not appreciably different from the one used in OSHA Method 1012 and the solution was injected into a GC-MS instead of a GC-ECD, the results indicated diacetyl and 2,3-pentanedione produced E- and Z-isomers of D-diacetyl and D-2,3-pentanedione in both aqueous and ethanol solutions. Also, the isomer peaks with highest abundance in the aqueous solution also had the highest abundance in ethanol solutions.

Table 4-4: p-Values to Compare Isomer AUC Ratios of D-Diacetyl

Method	Tenax-Water-Hexane Standard	O-Oxime Liquid/Solid Spiking	Ketone Liquid/Solid Spiking	Ketone Vapor/Solid Spiking
Tenax-Water-Hexane Standard		Not Applicable (N/A)	N/A	N/A
O-Oxime Liquid/Solid Spiking	0.0123*		N/A	N/A
Ketone Liquid/Solid Spiking	0.0001*	0.0010*		N/A
Ketone Vapor/Solid Spiking	< 0.0001*	< 0.0001*	0.2033	

*: Significantly different at $p \leq 0.05$

4.5 Selection of m/z in SIM

To determine the usefulness of m/z 240, Table 7-2 through Table 7-4 in the Appendix demonstrate the results of calculations for pure hexane, Tenax-hexane, and Tenax-water-hexane standards respectively relative to the behavior of SIM m/z ions with concentration. Table 4-5 provides the statistical summary of the three standards shown in Table 7-2 through Table 7-4. As shown in Table 4-5, % extracted AUC 240 and % calculated/observed AUC 181+240 remained constant throughout the observed M-acetoin concentration range within the pure hexane, Tenax-hexane, and Tenax-water-hexane standards because CVs were less than 10 %. However, % extracted AUC 240 slightly decreased as M-acetoin mass injected increased. Furthermore, extracted AUC 240 and % calculated/observed AUC 181+240 were statistically the same among the three standards. When a two-tailed Student t-test was performed assuming unequal variance at $\alpha = 0.05$, the p-values were greater than 0.05 for each combination of compared standards, that is, no statistical difference.

Since M-acetoin was quantifiable by extraction using m/z 240, SIM with m/z 181 and 240 together were used to quantify the mixture of ketones. Although % extracted AUC 240 remained the same within the same day, autotuning the GC-MS caused infrequent variance. Thus, % extracted AUC 240 was obtained daily when the mixture of ketones was analyzed at the target concentration of the sample because % extracted AUC 240 decreased as M-acetoin mass injected increased.

Table 7-5 in the Appendix shows the results of M-heptanal calculations for the pure hexane standard relative to the behavior of SIM m/z ions. The % extracted AUC 239 remained constant throughout the observed M-heptanal concentration range because the mean was 17.24 ± 0.68 % with a CV of 4.0 %. Also, % Calc/Obs AUC 181+239+240 indicates that the AUCs for the three ions are additive because the mean was 99.73 ± 0.68 % with a CV of 0.68 %.

Table 4-5: SIM Ion Behavior of M-acetoin for Three Standards

Method	Standard	Mean (%)	SD (%)	CV
% Extracted AUC 240	Pure Hexane	29.9	1.3	4.5
	Tenax-Hexane	30.0	1.8	5.8
	Tenax-Water-Hexane	30.3	1.5	5.1
% Calc/Obs AUC 181+240	Pure Hexane	100.68	0.23	0.23
	Tenax-Hexane	101.07	0.78	0.77
	Tenax-Water-Hexane	100.98	0.49	0.49

4.6 Quantification of PFBHA O-Oximes Using Different Standard Curves

Pure hexane standards were used for the preliminary study. When pure hexane standard curves for the 5 O-oximes were obtained by progressive dilution in hexane from a 1 mg/mL stock solution, the injected masses between 0.050 ng (except M-acetoin of 0.10 ng) and 200 ng were within less than 10 % CV. However, it was later determined that the quantification using

pure hexane standards was not efficient for lower concentration samples because the peak patterns were different with and without the PFBHA background.

When a hexane solvent blank was injected, the background baseline in the gas chromatogram was around 200 abundance. On the other hand, Tenax-hexane and Tenax-water-hexane method blanks had background baselines of 4000 abundance around the retention time for mono-substituted O-oximes and 2000 abundance around the retention time for di-substituted O-oximes. These different background baselines between the pure hexane standard and the actual samples with excess PFBHA gave different peak patterns for O-oximes, causing inconsistent peak integration relative to the pure hexane reference. However, when Tenax-hexane and Tenax-water-hexane standard curves were used to analyze samples, peaks for standards and actual samples had the same patterns, thus minimizing systematic errors of integration. Therefore, Tenax-hexane and Tenax-water-hexane standard curves were used for quantitation. The PFBHA O-oxime reference in hexane standardization was used to assess PFBHA O-oxime purity since there was no excess PFBHA and Tenax to complicate matters. The LQLs were lower for the Tenax-PFBHA systems because the method blank was already a high value well above the instrumental LQL with hexane alone.

Since the Tenax-water-hexane standard curves were used to quantify vapor samples, the simple linear regression equations and working linear ranges for each O-oxime are provided in Table 4-6. However, the standard curves and LQLs changed depending on GC-MS conditions necessitating daily standard curves with concentrations around the target sample concentrations.

The Tenax-hexane and Tenax-water-hexane standard solutions contained an excess of PFBHA. Thus, some M-diacetyl and M-2,3-pentanedione became di-substituted O-oximes with

slow reaction rate in hexane than in aqueous solutions. Therefore, the aliquot was injected within 5 min of the standard solution preparation.

The amounts of di-substituted O-oximes found in Tenax-hexane and Tenax-water-hexane standard solutions, when mono-substituted O-oximes were spiked into the hexane solution containing excess PFBHA within 5 min of the standard solution preparation, were less than the LQLs for both D-diacetyl and D-2,3-pentanedione.

Table 4-6: Tenax-Water-Hexane Internal Standard Curves

PFBHA O-Oxime	Regression Equation	R^2	n	p-Value	Working Linear Range
M-Acetoin	y = 0.0842x - 0.013	0.9974	9	< 0.001	0.275 - 13.2 ng
M-Diacetyl	y = 0.1699x + 0.388	0.9992	9	< 0.001	0.275 - 13.2 ng
M-2,3-Pentanedione	y = 0.0970x + 0.063	0.9991	9	< 0.001	0.287 - 13.8 ng
D-Diacetyl	y = 0.2458x + 0.040	0.9993	9	< 0.001	0.469 - 22.5 ng
D-2,3-Pentanedione	y = 0.2261x + 0.071	0.9993	9	< 0.001	0.473 - 22.7 ng

4.7 Development of Dynamic Sampling

4.7.1 Preliminary Study with Higher Target Concentrations

The ketone liquid spiking recoveries of acetoin and diacetyl exceeded 75 % using pure hexane standard curves when ketones were spiked into aqueous solution and onto solid sorbents. However, the recoveries of vapor acetoin and diacetyl were less than 75 % for most of the concentrations tested. The results also indicated that a 50 mL/min flow rate caused breakthrough through a 200 mg front section onto a 50 mg back section at 2.0 mg/m^3 8-hour TWA. Thus, a 10 mL/min flow rate was used for further analysis.

When different desorption durations were tested, the recoveries of acetoin vapor using the 200 mg coated Tenax TA at 16.0 mg/m^3 for 1 hour (equivalent to 2.0 mg/m^3 8-hour TWA) were 61.7 ± 3.7, 85.7 ± 8.3, 87.6 ± 11.2, and 96.2 ± 4.1 % for 12, 24, 48 and 72 hours desorption

time respectively at 25 ºC. Similarly for diacetyl vapor, the recoveries were 59.4 ± 2.6, 67.3 ± 3.7, 69.4 ± 3.0, and 69.6 ± 3.4 % respectively. Although the recoveries for the uncoated sorbent were similar to those for the coated sorbent, the coefficients of variation were usually higher than 25 % compared to less than 10 % for the coated sorbent. Thus, to obtain high recovery and precision using hexane alone, coated Tenax TA had to be desorbed by hexane for at least 24 hours for ≥ 75 % yield and 48 hours for maximal yield for acetoin, and at least 24 hours for maximal yield for diacetyl. For diacetyl, the recovery was lower than 70 % even after 72 hours. Furthermore, when the hexane solution was placed in a microwave, in an oven at 60 ºC from 1 hour to 72 hours, or in an ultrasonicator from 1 hour to 72 hours, diacetyl vapor recovery greater than 75 % was not achieved.

Table 4-7 summarizes the characteristics of the solvents tested. Chloroform, dichloromethane, tetrahydrofuran, and 1,4-dioxane were unusable because Tenax TA decomposed in the solvent. Hexane, pentane, cyclohexane, nitromethane, chloroform, dichloromethane were unsuitable because PFBHA was not very soluable. Methanol, ethanol, acetonitrile, nitromethane, and 1,4-dioxane were unsuitable solvents because O-oxime peaks in the GC chromatograms became multiple peaks. The O-oximes were unstable in the solvents or had issues with GC resolution compared to hexane. For instance, Figure 7-1 demonstrates a gas chromatogram using TIC when M-diacetyl was desolved in hexane for 3 days. There were E- and Z- isomer peaks of M-diacetyl at retention times of 10.7 and 11.2 min. Similarly, Figure 7-2 and Figure 7-3 demonstrate gas chromatograms using TIC when M-diacetyl was desolved in ethanol and acetonitrile solutions respectively for 3 days. The gas chromatograms show additional unkown peaks along with the two M-diacetyl peaks at the retention time. Since it was hard to find a simple solvent that solved all the problems, a water and hexane mixture was tested

to maximize the recovery. Water was a good solvent for PFBHA but a poor one for the O-oximes, and vice versa for hexane. Water also allowed un-reacted ketone (also water soluble) in the tubes to react once the solid sorbent was suspended in water. From the synthesis study, ketones reacted to at least a mono-derivative within one hour in water. Since O-oximes were non-polar, O-oximes could be extracted by hexane and would not remain in water thus enhancing reaction completion. This method was further tested using new target concentrations (0, 1, 5, 10, and 20 ppb at 8-hour TWA) and recovery results were compared to the desorption method solely utilizing hexane. Furthermore, 2,3-pentanedione was added to the list of ketones as the major substitute for diacetyl.

Table 4-7: Solvent Characteristics for PFBHA O-Oximes and Tenax TA stability and PFBHA solubility at 25 °C

Solvent	PFBHA O-Oxime Stability or GC Resolution			Tenax TA Stability	PFBHA Solubility
	M-acetoin	M-diacetyl	D-diacetyl		
Hexane	Yes	Yes	Yes	Yes	No
Pentane	Yes	Yes	Yes	Yes	No
Cyclohexane	Yes	Yes	Yes	Yes	No
Methanol	No	No	No*	Yes	Yes
Ethanol	No	No	No*	Yes	Yes
Acetonitrile	No	No	No*	Yes	Yes
Nitromethane	No	No	No*	Yes	No
Chloroform	Yes	Yes	Yes	No	No
Dichloromethane	Yes	Yes	Yes	No	No
Tetrahydrofuran	Yes	Yes	Yes	No	Yes
1,4-Dioxane	No	No	No	No	No

*: Minor effects

4.7.2 PFBHA O-Oxime Liquid/Solid Spiking

Table 7-6 and Table 7-7 in the Appendix summarize the O-oxime liquid/solid spiking recoveries for Tenax-hexane and Tenax-water-hexane desorption methods respectively. Table

4-8 compares O-oxime liquid/solid spiking recoveries from 1 to 20 ppb for both Tenax-hexane and Tenax-water-hexane methods. For M-acetoin and D-diacetyl, there was no significant difference in recovery when Tenax TA was desorbed in hexane or when Tenax TA was desorbed in water and O-oximes were extracted in hexane at $p \leq 0.05$. However, D-2,3-pentanedione had significantly higher recovery when Tenax TA was desorbed in water and O-oximes were extracted in hexane. The recoveries were still higher than 94 % for both systems and agree within 2-3 %. Overall, this result shows that O-oximes spiked onto Tenax TA in aqueous solution could be extracted efficiently by one extraction, and that the desorption step was efficient once the PFBHA O-oximes had formed.

Table 4-8: Summary of Pooled Data for PFBHA O-Oxime Liquid/Solid Spiking Recoveries (%) over the 1-20 ppb Ketone Equivalent Range

PFBHA O-Oxime	Method	n	Mean	SD	CV	p-Value
M-Acetoin	Tenax-Hexane	12	95.8	3.5	3.7	0.9212
	Tenax-Water-Hexane	12	96.0	5.0	5.2	
D-Diacetyl	Tenax-Hexane	12	95.5	3.9	4.0	0.1037
	Tenax-Water-Hexane	12	98.2	4.2	4.2	
D-2,3-Pentanedione	Tenax-Hexane	12	94.0	3.1	3.3	0.0183*
	Tenax-Water-Hexane	12	97.2	3.1	3.2	
PFBHA O-Oximes	Tenax-Hexane	36	95.1	3.5	3.7	0.0253*
	Tenax-Water-Hexane	36	97.2	4.1	4.3	

*: Significantly different at $p \leq 0.05$

4.7.3 Ketone Liquid/Solid Spiking

Table 7-8 and Table 7-9 in the Appendix summarize ketone liquid/solid spiking recoveries for Tenax-hexane and Tenax-water-hexane desorption methods respectively. Table 4-9 compares ketone liquid/solid spiking recoveries from 1 to 20 ppb pooled for both Tenax-hexane and Tenax-water-hexane methods. The recovery for acetoin liquid/solid spiking was

equivalent for the Tenax-hexane and Tenax-water-hexane methods. However, the recovery for diacetyl and 2,3-pentanedione liquid/solid spiking with the Tenax-water-hexane method gave statistically higher recovery than the Tenax-hexane method.

Table 4-10 compares the recoveries for O-oxime and ketone spiking. The recovery for diacetyl and 2,3-pentanedione liquid/solid spiking with the Tenax-hexane method had significantly lower recoveries compared to the O-oxime liquid/solid spiking. On the other hand, all ketone spiking recoveries were equivalent to O-oxime spiking with the Tenax-water-hexane method. Thus, the Tenax-water-hexane desorption/extraction method was used for further analysis including vapor sampling.

Table 4-9: Summary of Pooled Data for Ketone Liquid/Solid Spiking Recoveries (%) over the 1-20 ppb Ketone Equivalent Range

Ketone	Method	n	Mean	SD	CV	p-Value
Acetoin	Tenax-Hexane	12	94.8	9.2	9.7	0.5103
	Tenax-Water-Hexane	12	92.4	8.3	9.0	
Diacetyl	Tenax-Hexane	12	71.1	4.2	5.9	< 0.0001*
	Tenax-Water-Hexane	12	94.6	9.0	9.6	
2,3-Pentanedione	Tenax-Hexane	12	60.2	2.9	4.8	< 0.0001*
	Tenax-Water-Hexane	12	92.5	8.1	8.8	

*: Significantly different at $p \leq 0.05$

Table 4-10: Comparison of PFBHA O-Oxime Liquid/Solid Spiking and Ketone Liquid/Solid Spiking Recoveries (%)

Method	Compound	n	Mean	SD	CV	p-Value
Tenax-Hexane	M-Acetoin	12	95.8	3.5	3.7	0.7079
	Pure Acetoin	12	94.8	9.2	9.7	
	D-Diacetyl	12	95.5	3.9	4.0	< 0.0001*
	Pure Diacetyl	12	71.1	4.2	5.9	
	D-2,3-Pentanedione	12	94.0	3.1	3.3	< 0.0001*
	Pure 2,3-Pentanedione	12	60.2	2.9	4.8	
Tenax-Water-Hexane	M-Acetoin	12	96.0	5.0	5.2	0.2076
	Pure Acetoin	12	92.4	8.3	9.0	
	D-Diacetyl	12	98.2	4.2	4.2	0.2302
	Pure Diacetyl	12	94.6	9.0	9.6	
	D-2,3-Pentanedione	12	97.2	3.1	3.2	0.0828
	Pure 2,3-Pentanedione	12	92.5	8.1	8.8	

*: Significantly different at $p \leq 0.05$

4.7.4 Reaction Time Using Ketone Liquid/Liquid Spiking

Table 7-10 in the Appendix summarizes ketone liquid/liquid spiking recoveries for the Tenax-water-hexane method over 3 days desorption/reaction time at 10 ppb equivalent. The table shows the equivalent ketone recoveries from mono- and di- substituted O-oximes as well as their totals. Both diacetyl and 2,3-pentanedione did not completely turn into di-derivatives. Since the reaction was not completed in 3 days and summing mono- and di- substituted O-oximes gave statistically the same recoveries for the 1-hour liquid/solid spiking and the 3-day liquid/liquid spiking, the Tenax-water-hexane 1-hour desorption/extraction method was used for further analysis. The equivalent ketone recoveries from di- substituted O-oximes were higher for vapor/solid spiking than liquid/liquid spiking even with a one hour desorption/reaction time because most ketones reacted with PFBHA during vapor sampling.

4.7.5 Calibration of Thermometer and Hygrometer

Table 7-11 in the Appendix shows the temperature data used to calibrate the thermometer of the multi-function thermal anemometer and to assess the setting of the environmental chamber. The calibration equation was $y = 1.0022x - 0.0505$ with R^2 of 0.99997 and p-value of < 0.001. The intercept (-0.0505) of the linear regression equation had no significant difference from zero, and the slope (1.0022) had no significant difference from one at $p \leq 0.05$. Since the multi-function thermometer operated accurately, the actual reading was used instead of the calibration equation. The environmental chamber was set at 3.0, 23.7, and 38.7 ºC to create 5, 25, and 40 ºC.

Table 7-12 in the Appendix shows the expected and observed RH data used to calibrate the hygrometer. The calibration equation was $y = 0.8752x + 1.8914$ with R^2 of 0.9976 and p-value of < 0.001. Since the slope (0.8752) was statistically different from one at $p \leq 0.05$, the calibration equation was used to calculate the actual humidity generated during the experiments.

4.7.6 Comparison of Individual Ketone Vapors and Their Vapor Mixture

Table 7-13 in the Appendix compares the recoveries when ketones were sampled in triplicate individually or as a mixture at 80 ppb at 25 ºC at 5 % RH for 1 hour at 100 mL/min. There was no statistical difference in individual vapor or mixtures for acetoin and 2,3-pentanedione, and mean recoveries were above 93.1 %. On the other hand, diacetyl vapor recovery was statistically different when sampled individually or as a mixture. However, the difference was only 6.4 %, and both mean recoveries exceeded 87.7 %.

4.7.7 Comparison of Vapor Sampling Time

Table 7-14 in the Appendix compares the recoveries when ketones were sampled in triplicate as a mixture at 25 ºC, 5 % RH, and 100 mL/min at either 80 ppb for 1 hour or 10 ppb

for 8 hours. There was no statistical difference in acetoin and diacetyl vapor recoveries independent of time, and mean recoveries were above 87.7 %. On the other hand, 2,3-pentanedione vapor recovery was statistically different when sampled at different durations. However, both mean recoveries exceeded 80.5 %.

4.7.8 Comparison of RH during Vapor Sampling

Table 7-15 through Table 7-18 in the Appendix compare the recoveries when ketones were sampled in triplicate as a mixture at 25 ºC and 100 mL/min for 8 hours at either 5 or 80 % RH for 1, 5, 10, and 20 ppb respectively. There was no statistical difference among recoveries at any concentration for a particular ketone at any RH. The recoveries were acceptable at 5, 10, and 20 ppb because all of the means exceeded 78.6 % with less than 10 % CV. However, most of the ketone sampling efficiencies were not acceptable at 1 ppb because the CVs were over 10 % and percent relative errors were over 25 %. Thus, 1 ppb was below the LQL of the method.

Since the recoveries at 5, 10, and 20 ppb were independent of RH with acceptable accuracy and precision, the data were pooled for each RH both individually and combined except for the 1 ppb datain Table 4-11. The mean recoveries for all three ketones exceeded 75 % with the CVs being less than 10 %.

Table 4-11: Pooled Vapor Sampling Recoveries (%) at 5, 10, and 20 ppb at Different RH and 25 °C

Ketone	RH (%)	n	Mean	SD	CV
Acetoin	5	9	94.1	6.4	6.8
	80	9	86.3	5.1	5.9
	5 and 80	18	90.2	6.9	7.7
Diacetyl	5	9	89.6	6.6	7.3
	80	9	94.8	3.8	4.0
	5 and 80	18	92.2	5.9	6.4
2,3-Pentanedione	5	9	82.1	5.0	6.1
	80	9	82.9	3.9	4.7
	5 and 80	18	82.5	4.4	5.3

4.7.9 Comparison of Temperature during Vapor Sampling

Table 7-19 and Table 7-20 in the Appendix compare the recoveries when ketones were sampled in triplicate as a mixture at 20 ppb, 25 ºC, and 100 mL/min for 8 hours at 5 and 80 % RH respectively. Table 4-12 summarizes the results in Table 7-19 and Table 7-20 by providing the p-values for the corresponding t-tests. At both 5 and 80 % RH, the acetoin mean recoveries at 5 ºC were statistically different at 25 and 40 ºC. Also, the acetoin mean recoveries were below 75 % at 5 ºC at both 5 % and 80 % RH.

There was no statistically significant difference in each ketone vapor recovery at 5 % RH among all three temperatures with the exception of acetoin at 5 ºC, and the mean recoveries were above 77.9 %. However, there was a statistically significant difference in diacetyl vapor recovery at 80 % RH among all three temperatures, and the diacetyl mean recoveries increased as temperature increased. Although the diacetyl mean recoveries ranged from 87.0 to 102.6 % among the three temperatures, the mean recoveries remained within the 75 to 125 % acceptable range from the NIOSH criteria.[148]

The 2,3-pentanedione mean recoveries also increased as temperature increased at 80 % RH. In fact, there was no statistical difference between 5 and 25 ºC, or 25 and 40 ºC, but there was statistical difference between 5 and 40 ºC. However, the 2,3-pentanedione mean recoveries ranged from 78.5 to 88.3 % among the three temperatures.

Table 4-12: p-Values to Compare Dependence of Recoveries on Temperature at Constant RH

Ketone	RH (%)	5 vs 25 ºC	5 vs 40 ºC	25 vs 40 ºC
Acetoin	5	0.0173*	0.0092*	0.1404
	80	0.0077*	0.0123*	0.1184
Diacetyl	5	0.5234	0.4799	0.9110
	80	0.0336*	0.0091*	0.0142*
2,3-Pentanedione	5	0.5330	0.1413	0.3520
	80	0.0760	0.0390*	0.4906

*: Significantly different at $p \leq 0.05$

4.7.10 Sampling Capacity

No breakthrough of the front tube was confirmed at 1, 5, 10, and 20 ppb at 5 and 80 % RH at 100 mg/mL for 8-hour sampling at 25 °C using two tubes connected in series (200/200 mg). Figure 4-7 shows the percent vapor collected in front, back, and total (front and back) tubes when acetoin vapor from 25.6 ppm to 160 ppm at 5 % RH was sampled at 100 mL/min for 1 hour. The critical PFBHA:acetoin molar ratio at 75 % capacity was extrapolated from the trendline equation, and determined as 5.0:1. At 75 % recovery for acetoin, the molar ratio was equivalent to 16 µmol of acetoin, so 12 µmol were held in the front tube. Thus, 66 ppm of acetoin could be sampled at 100 mL/min for 1 hour with over 75 % recovery. If the sampling efficiency remained the same for longer sampling time, the concentration was also equivalent to 8.2 ppm at 100 mL/min for 8-hour sampling.

Figure 4-8 shows the percent vapor collected in front, back, and total tubes when diacetyl vapor from 3.2 ppm to 24 ppm at 5 % RH was sampled at 100 mL/min for 1 hour. The critical PFBHA:diacetyl molar ratio at 75 % capacity was extrapolated as 15:1. At 75 % recovery for diacetyl, the molar ratio was equivalent to 5.4 µmol of diacetyl, so 4.0 µmol were held in the

front tube. Thus, 22 ppm of diacetyl at 100 mL/min for 1 hour, which was also equivalent to 2.7 ppm at 100 mL/min for 8-hour sampling, could be sampled with over 75 % recovery.

Figure 4-9 shows the percent vapor collected in front, back, and total tubes when 2,3-pentanedione vapor from 8 ppm to 80 ppm at 5 % RH was sampled at 100 mL/min for 1 hour. The critical PFBHA:2,3-pentanedione molar ratio at 75 % capacity was extrapolated as 3.6:1. At 75 % recovery for 2,3-pentanedione, the molar ratio was equivalent to 22 µmol of 2,3-pentanedione, so 17 µmol were held in the front tube. Thus, 91 ppm of 2,3-pentanedione at 100 mL/min for 1 hour, which was also equivalent to 11 ppm at 100 mL/min for 8-hour sampling, could be sampled with over 75 % recovery.

Figure 4-10 and Figure 4-11 show the percent vapor collected in front, back, and total tubes when three ketone vapors as a mixture from 2.4 ppm (0.8 ppm of each ketone) to 72 ppm (24 ppm of each ketone) at 5 and 80 % RH respectively were sampled at 100 mL/min for 1 hour. The concentrations where about 25 % breakthrough occurs for at least one ketone were determined for both 5 and 80 % RH. Diacetyl experienced most breakthrough, and the critical PFBHA:diacetyl molar ratio at 75 % capacity was extrapolated as 55:1 and 18:1 at 5 and 80 % RH respectively.

At 75 % recovery for the ketone mixture at 5 % RH, the molar ratio was equivalent to 1.5 µmol of diacetyl, so 1.1 µmol of diacetyl were held in the front tube. Thus, 6.0 ppm of diacetyl at 100 mL/min for 1 hour, which was also equivalent to 0.75 ppm at 100 mL/min for 8-hour sampling, could be sampled with over 75 % recovery along with the same concentration of acetoin and 2,3-pentanedione with over 95 % recovery. Therefore, approximately 4.5 µmol of ketones (1.5 µmol of each ketone) could be held in the front tube.

At 75 % recovery for the ketone mixture at 80 % RH, the molar ratio was equivalent to 4.4 µmol of diacetyl, so 3.3 µmol of diacetyl were held in the front tube. Thus, 18 ppm of diacetyl at 100 mL/min for 1 hour, which was also equivalent to 2.2 ppm at 100 mL/min for 8-hour sampling, could be sampled with over 75 % recovery along with the same concentration of acetoin and 2,3-pentanedione with over 75 % recovery. Therefore, approximately 13 µmol of ketones (4.4 µmol of each ketone) could be held in the front tube.

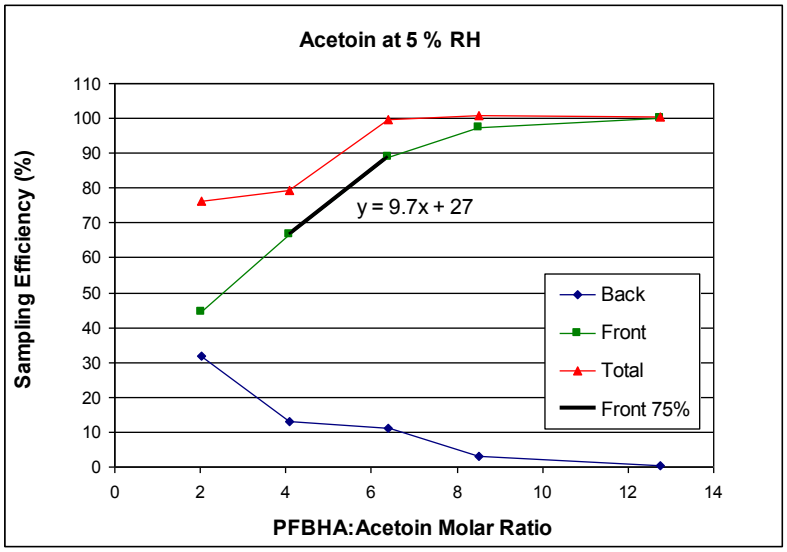


Figure 4-7: Tube Sampling Capacity for Acetoin Vapor at 5 % RH and 25 °C

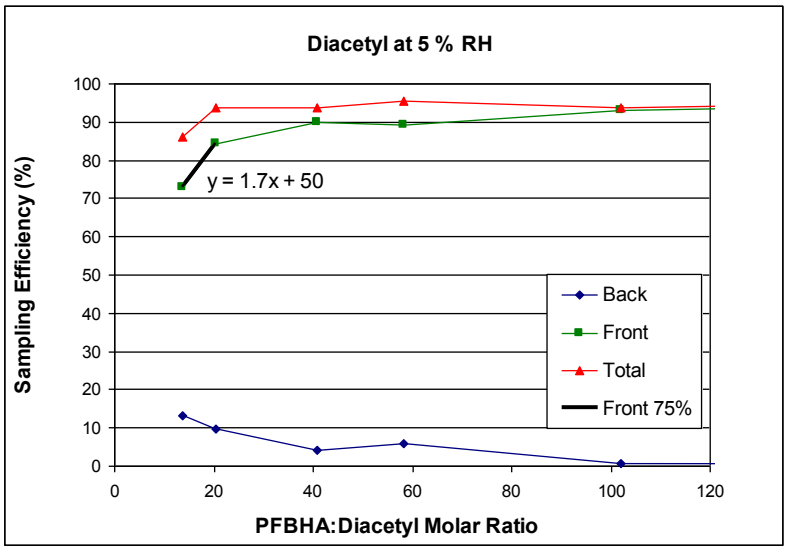


Figure 4-8: Tube Sampling Capacity for Diacetyl Vapor at 5 % RH and 25 °C

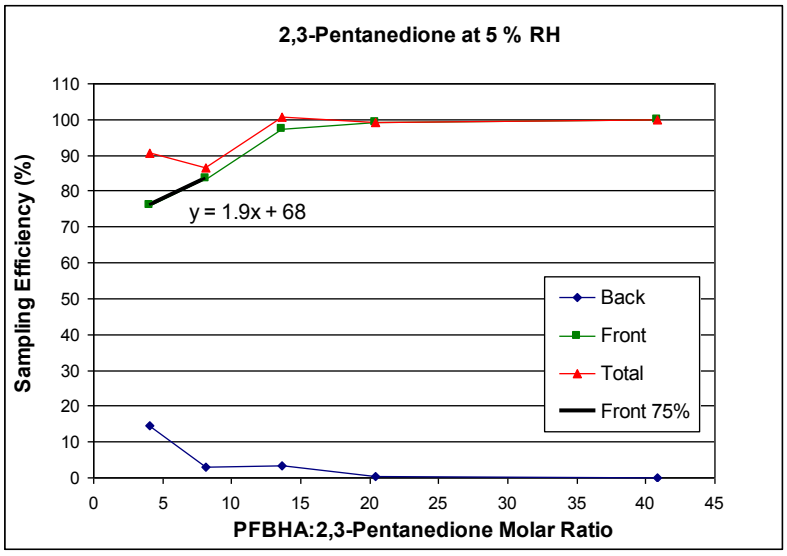


Figure 4-9: Tube Sampling Capacity for 2,3-Pentanedione Vapor at 5 % RH and 25 °C

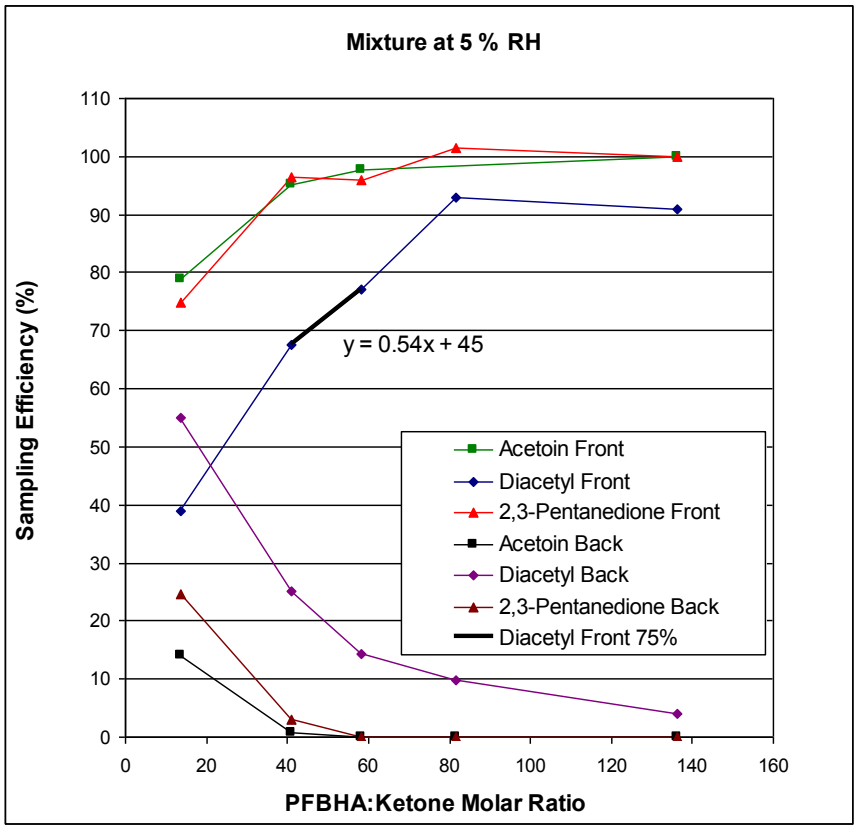


Figure 4-10: Tube Sampling Capacity for the Vapor Mixture at 5 % RH and 25 °C

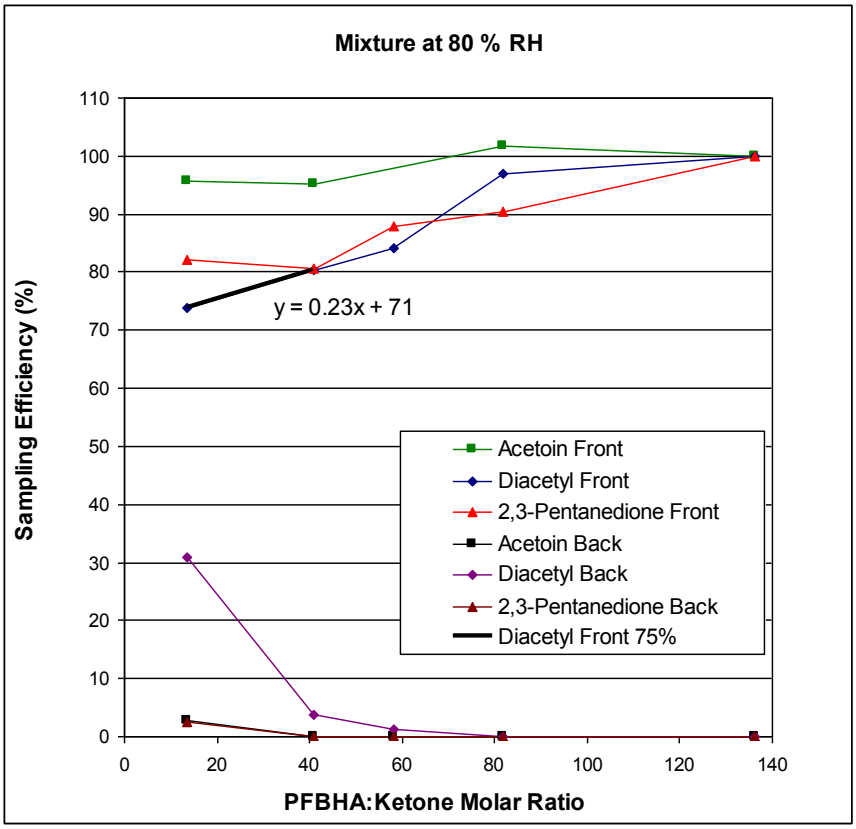


Figure 4-11: Tube Sampling Capacity for the Vapor Mixture at 80 % RH and 25 °C

4.7.11 Determination of Dynamic Sampling Tube Storage Periods after Sampling

Table 7-21 and Table 7-22 in the Appendix indicate the acetoin sample recoveries when vapor mixtures at 80 ppb and 25 ºC at 5 and 80 % RH respectively were sampled for 1 hour at 100 mL/min, and the tubes were stored at different temperature and duration combinations. The p-values indicate whether the recoveries were statistically different from the immediate analysis baseline (0 day storage). All the samples maintained statistically the same recoveries at all the tested storage temperature and duration combinations except when the tubes were stored for 30 days at either -20 or 5 ºC after sampling at 80 % RH. However, all of the mean recoveries were above 77.9 %.

Table 7-23 and Table 7-24 in the Appendix indicate the diacetyl sample recoveries when vapor mixtures at 80 ppb and 25 ºC at 5 and 80 % RH respectively were sampled for 1 hour at 100 mL/min. All the samples maintained statistically the same recoveries at all the tested storage temperature and duration combinations except when the tubes were stored for 30 days at 5 ºC after sampling at 80 % RH. All the samples maintained above 81.3 % mean recoveries at all the tested storage temperature and duration combinations except when the tubes were stored for 30 days at 5 ºC after sampling at both 5 and 80 % RH. However, this low recovery was due to an abnormally high blank. There was a large peak around the M-diacetyl retention time, so subtracting the blank AUC from the M-diacetyl AUC generated a result less than the LQL. Thus, the systematic errors caused low recoveries as the diacetyl recoveries were calculated solely from to D-diacetyl.

Table 7-25 and Table 7-26 in the Appendix indicate the 2,3-pentanedione sample recoveries when vapor mixtures at 80 ppb and 25 ºC at 5 and 80 % RH respectively were sampled for 1 hour at 100 mL/min. All the samples maintained statistically the same recoveries at all the tested storage temperature and duration combinations except when the tubes were stored for 3 days at 5 ºC and 30 days at either 5 or 25 ºC after sampling at 5 % RH and except when the tubes were stored for 30 days at 5 ºC after sampling at 80 % RH. However, all of the mean recoveries were above 78.8 %.

Table 4-13 summarizes the recoveries at different storage temperature conditions when RH during sampling was 5 or 80 % after dropping the diacetyl data stored for 30 days at 5 ºC. There was no statistical difference for diacetyl and 2,3-pentanedione when 5 and 80 % RH were compared, but acetoin demonstrated statistical difference. However, the NIOSH collected sample stability criterion of 30 days was met because the mean recoveries were above 75 % for

all three ketones at any storage temperature. Thus, the sampling tubes do not require refrigeration after sampling around the 10 ppb 8-hours TWA.

Although overall recoveries for diacetyl and 2,3-pentanedione remained the same at the different storage temperatures and durations, the ratio of mono- and di-substituted O-oximes were different as shown in Table 7-27 through Table 7-30 in the Appendix. As the storage period became longer, the amount of the di-substituted O-oximes became larger. This result indicated that the ketones or mono-substituted O-oximes kept reacting inside of the solid sorbent tubes during storage. Also, the reaction rate was faster at the higher storage temperatures.

Table 4-13: Pooled Sample Recoveries (%) of 80 ppb at 25 ºC and 5 or 80 % RH Sampled for 1 Hour at 100 mL/min

Ketone	Sampling RH (%)	n	Mean	SD	CV	p-Value
Acetoin	5	21	93.9	7.2	7.6	< 0.0001*
	80	21	84.6	4.8	5.7	
Diacetyl	5	18	85.3	5.8	6.7	0.8292
	80	18	85.8	6.8	7.9	
2,3-Pentanedione	5	21	85.1	6.2	7.3	0.1632
	80	21	82.8	3.8	4.6	

*: Significantly different at $p \leq 0.05$

4.8 Development of Passive Sampling

4.8.1 Preliminary Study with Mini-Passive Samplers

Mini-passive samplers were initially used to test acetoin, diacetyl, and 2,3-pentanedione at 0, 0.1, 0.5, 1.0, 1.5, and 2.0 mg/m^3 (0, 28, 139, 277, 416, and 555 ppb of acetoin, 0, 28, 142, 284, 426, and 568 ppb of diacetyl, 0, 24, 122, 244, 366, and 489 ppb of 2,3-pentanedione respectively) at 25 ºC. However, the mini-passive samplers had theoretical and experimental

sampling constants that were not sensitive enough for 2012 ACGIH TLV-TWA for diacetyl. Thus, further study with mini-passive samplers was abandoned.

4.8.2 Development of Custom Passive Samplers

To produce pellets, 200 mg of the PFBHA coated Tenax TA at pressures of two to eight tons was first used to make 30 mm diameter pellets to be consistent with the dynamic sampling tubes and the mini-pellets. However, 200 mg was not enough solid sorbent to create pellets. Experimentation showed that 300 mg was the smallest amount that could produce a stable pellet that would not break or crack. Two tons of pressure produced no pellet, and four tons produced a pellet occasionally, but not consistently. Six tons of pressure produced pellets most of the time without breaking. Table 4-14 provides the dimensions of the custom developed passive sampler when 300 mg with six tons of pressure were used to create the pellets. The data were from the first 90 samplers that did not break and were used later for actual sampling and analysis. The experimental arithmetic mean path length was used to calculate the theoretical sampling constants for acetoin, diacetyl, and 2,3-pentanedione.

Table 4-14: Dimensions of the Custom Developed Passive Samplers

	Mean	SD	CV	n
Pellet Weight (mg)	300.6	2.9	0.96	90
Pellet Thickness (mm)	0.533	0.017	3.1	90
Diffusion Path Length (mm)	3.23	0.11	3.5	90
Pellet Diameter (mm)	29.97	0.02	0.07	4

4.8.3 Calibration of Anemometer and Syringe Pump

Table 7-31 in the Appendix shows the data used to calibrate the anemometer. The calibration equation was $y = 1.0512x - 0.1597$ with R^2 of 0.9962 and p-value of < 0.001. The intercept (-0.1597) of the linear regression equation had no significant difference from zero, and

the slope (1.0512) had no significant difference from one at $p \leq 0.05$. Thus, the actual air velocity measurements read on the anemometer were used instead of the calibration equation.

Table 7-32 and Table 7-33 in the Appendix show the data used to calibrate the syringe pump using a 5-mL and 50-mL gas tight syringe respectively. The calibration equation for the 5-mL gas tight syringe was $y = 1.0043x - 0.0011$ with R^2 of 0.99998 and p-value of < 0.001. The intercept (-0.0011) of the linear regression equation had no significant difference from zero, and the slope (1.0043) had no significant difference from one at $p \leq 0.05$.

The calibration equation for the 50-mL gas tight syringe was $y = 0.9536x + 0.1041$ with R^2 of 0.9997 and p-value of < 0.001. Although the intercept (0.1041) of the linear regression equation had no significant difference from zero, the slope (0.9536) was statistically different from one at $p \leq 0.05$. Thus, the calibration equation was used to calculate the volume of aqueous solution generated in the exposure chamber. The aqueous solution weight before and after the experiment was obtained to confirm that the target ketone concentration and RH were generated by pumping the corresponding amount of aqueous solution.

4.8.4 PFBHA O-Oxime Liquid/Solid Spiking

Table 7-34 in the Appendix summarizes the O-oxime liquid/solid spiking recoveries of the custom developed pellets using the Tenax-water-hexane desorption method. Since all mean recoveries for each ketone at all the evaluated concentrations were above 84.8 %, all of the data were pooled for each ketone. Table 4-15 shows the pooled data for each PFBHA O-oxime liquid/solid spiking recoveries over the 1-20 ppb ketone equivalent range. The mean recoveries for the ketones were above 85.5 % with less than 10 % CV. Thus, O-oximes spiked onto the

pellet could be desorbed and extracted by hexane from the aqueous solution accurately and precisely, and one extraction was sufficient.

Table 4-15: Summary of Pooled Data for PFBHA O-Oxime Liquid/Solid Spiking Recoveries (%) over the 1-20 ppb Ketone Equivalent Range

O-Oxime	n	Mean	SD	CV
M-Acetoin	12	86.9	4.1	4.7
D-Diacetyl	12	85.5	2.9	3.4
D-2,3-Pentanedione	12	95.3	5.1	5.3

4.8.5 Ketone Liquid/Solid Spiking

Table 7-35 in the Appendix summarizes the ketone liquid/solid spiking recoveries of the custom developed pellet using the optimized Tenax-water-hexane desorption method. Apart from the 1 ppb equivalent level where the mean recoveries were greater than 125 % for diacetyl and 2,3-pentanedione, all the mean recoveries were between 75 to 125 %. All the CVs were less than 10 % except for diacetyl at 1 ppb. Thus, the data for 1 ppb were excluded. Table 4-16 shows the pooled data for ketone liquid/solid spiking recoveries over the 5-40 ppb ketone equivalent range. The mean recoveries for the ketones were above 82.4 % with less than 10 % CV.

Table 4-17 compares PFBHA O-oxime liquid/solid spiking and ketone liquid/solid spiking recoveries using a Student t-test. Diacetyl recovery was statistically the same for the PFBHA O-oxime and ketone liquid/solid spiking. However, acetoin and 2,3-pentanedione showed significant differences. However, all of the recoveries were above 75 % with CVs less than 10 % as required via NIOSH quality assurance and quality control criteria.

Table 4-16: Summary of Pooled Data for Ketone Liquid/Solid Spiking Recoveries (%) over the 5-40 ppb Ketone Equivalent Range

Ketone	n	Mean	SD	CV
Acetoin	15	82.4	4.0	4.9
Diacetyl	15	90.4	8.9	9.8
2,3-Pentanedione	15	82.5	4.5	5.5

Table 4-17: Comparison of PFBHA O-Oxime Liquid/Solid Spiking and Ketone Liquid/Solid Spiking Recoveries (%)

Compound	n	Mean	SD	CV	P-value
M-Acetoin	12	86.9	4.1	4.7	0.0079*
Pure Acetoin	15	82.4	4.0	4.9	
D-Diacetyl	12	85.5	2.9	3.4	0.0612
Pure Diacetyl	15	90.4	8.9	9.8	
D-2,3-Pentanedione	12	95.3	5.1	5.3	< 0.0001*
Pure 2,3-Pentanedione	15	82.5	4.5	5.5	

*: Significantly different at $p \leq 0.05$

4.8.6 RH and Concentration in Exposure Chamber

Figure 4-12 shows the RH over time when dry air was generated. The RH in the exposure chamber reached 0.2 %, which indicated that the air was dry enough to use without Drierite to remove water vapor. The half-life ($t_{1/2}$) was determined by the time taken for the observed RH to reach halfway to the asymptote after the first RH observation. The observed $t_{1/2}$ value was about 3.5 min.

Figure 4-13 shows the RH over time to create 5 % RH. After ensuring steady state, the lid of chamber was opened 45 minutes later for 10 seconds, and it took about 15 minutes for the environment to return to 5 % RH. The observed $t_{1/2}$ values were about 3.5 min when starting dry air and water generation, and 2 min after opening the lid of the chamber.

Figure 4-14 shows the RH over time to create 80 % RH. The lid of chamber was opened 55 minutes later for 10 seconds, and it took about 15 minutes for the environment to return to

80 % RH. The observed $t_{1/2}$ values were about 3 min when starting dry air and water generation and 3.5 min after opening the lid of the chamber.

Adsorption of ketones on the Teflon tubing and mixing chamber inner walls was noted, because there were PFBHA O-oxime peaks from acetoin, diacetyl, and 2,3-pentanedione from the GC-MS analysis for both sites. This indicated that the actual concentrations generated in the exposure chamber were lower than the target concentrations. Furthermore, since the RH changed when opening the lid, it also indicated that the concentration of ketones would also decrease with about the same kinetic $t_{1/2}$ assumed. Thus, the optimized dynamic air sampling method was utilized to determine the actual ketone concentration in the chamber after correcting for recovery.

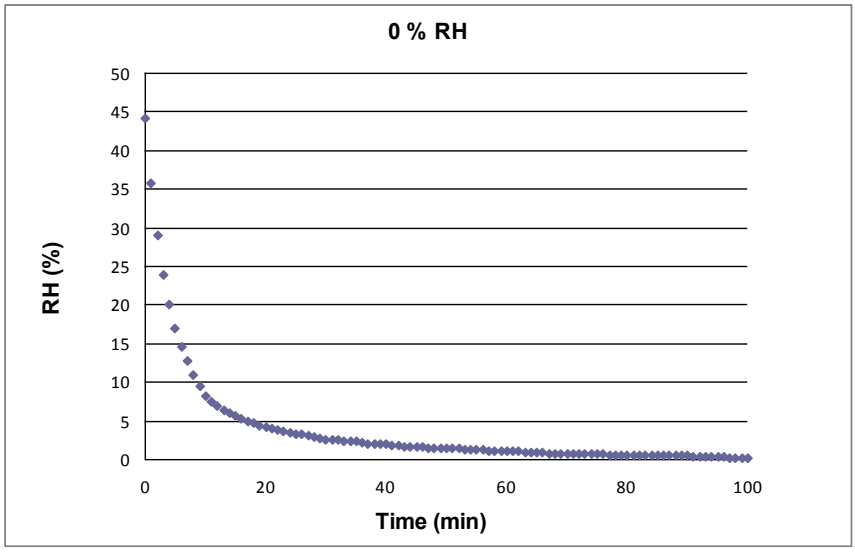


Figure 4-12: RH over Time at 0 % Target RH at 25 °C

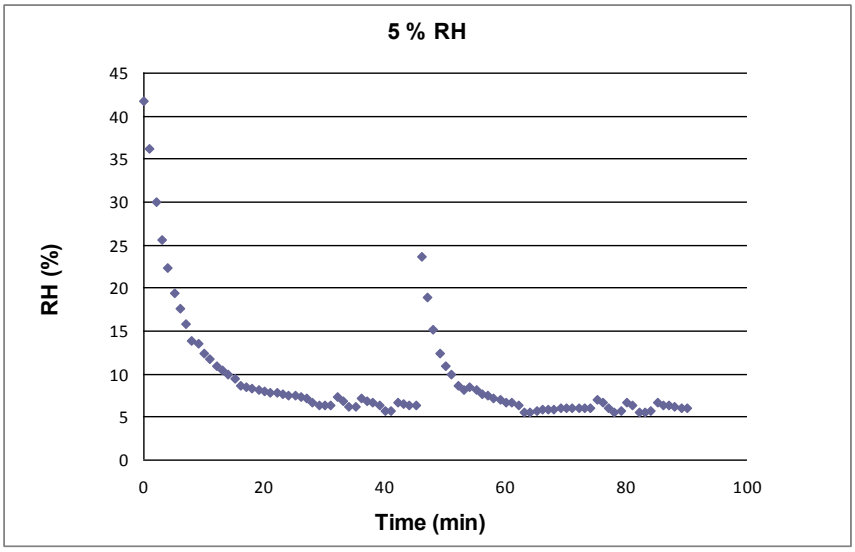


Figure 4-13: RH over Time at 5 % Target RH with Lid Opened at 45 Minutes at 25 °C

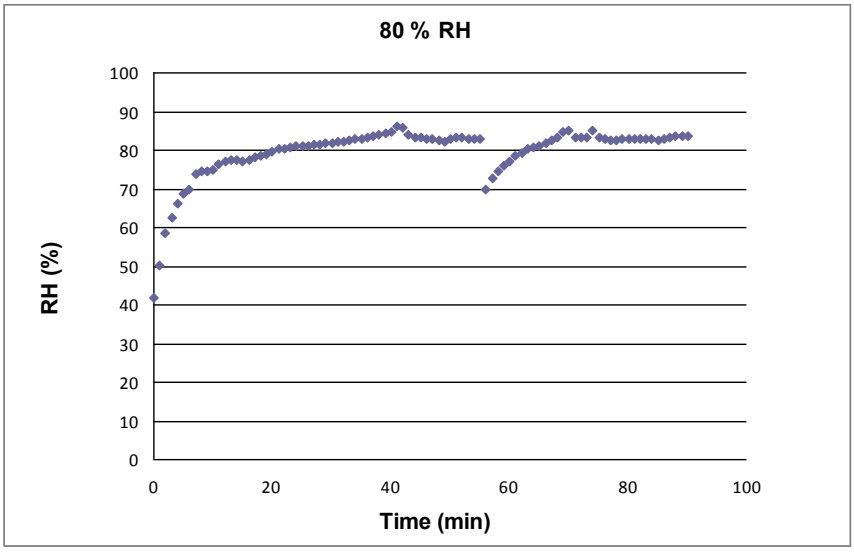


Figure 4-14: RH over Time at 80 % Target RH with Lid Opened at 55 Minutes at 25 °C

4.8.7 Experimental Sampling Constants for Temperature and RH Effects

Figure 7-4 through Figure 7-7 in the Appendix demonstrate the acetoin experimental sampling constants by providing a simple linear regression equation when ketones as a mixture were sampled at target concentrations of 0, 5, 10, 20, 30, and 40 ppb for 8-hour TWA equivalents at 25 and 40 ºC as well as at 5 and 80 % RH. Similarly, Figure 7-8 through Figure

7-11 and Figure 7-12 through Figure 7-15 in the Appendix demonstrate the experimental sampling constants for diacetyl and 2,3-pentanedione respectively. The slope indicates the experimental sampling constant assuming no back diffusion and no other systematic errors. It is uncorrected for actual pellet desorption efficiency.

Table 4-18 summarizes the ketone sampling constants from Figure 7-4 through Figure 7-15 to compare temperature and humidity effects. A two-tailed Student t-test and equation 3.8 were used to compare the slopes (experimental sampling constants) within each ketone for the different temperature and RH combinations. Similarly, it was determined whether each intercept was significantly different from zero. All of the intercepts were not significantly different from zero except for diacetyl at 40 ºC for both 5 and 80 % RH as well as for 2,3-pentanedione at 40 ºC and 5 % RH. Thus, the blank data representing zero were dropped from these three regressions. Then, the regression line was refit without the blank as shown in Table 4-18.

At 25 ºC, the acetoin, diacetyl, and 2,3-pentanedione sampling constants had no statistical difference at 5 and 80 % RH at $p \leq 0.05$. However, at 40 ºC, the acetoin, diacetyl, and 2,3-pentanedione sampling constants were statistically different at 5 and 80 % RH at $p \leq 0.05$. At 5 % RH, the acetoin, diacetyl, and 2,3-pentanedione sampling constants had no statistical difference at 25 and 40 ºC at $p \leq 0.05$. At 80 % RH, the acetoin, diacetyl, and 2,3-pentanedione sampling constants were statistically different at 25 and 40 ºC at $p \leq 0.05$. Overall, the sampling constants at both 40 ºC and 5 % RH were statistically lower for acetoin, diacetyl, and 2,3-pentanedione.

Table 4-19 provides the lowest detected ketone vapor concentrations using the custom developed passive samplers at 25 and 40 ºC for both 5 and 80 % RH where the CVs of mass collected on the pellets in triplicate were still less than 10 %. The concentrations of the actual

vapors were determined by the optimized dynamic sampling method corrected for recovery where the CVs of the concentrations in triplicate were less than 10 %. Among all combinations, the custom pellet collected ketones accurately and precisely at less then 10 ppb.

Table 4-18: Summary of Regression Equation at Different Temperatures and RHs

Ketone	Temp. (ºC)	RH (%)	Slope ± SD (mL/min)	Intercept ± SD (μg)
Acetoin	25	5	58.8 ± 8.0	0.09 ± 0.23
	25	80	60.4 ± 9.4	0.15 ± 0.26
	40	5	51.3 ± 5.8*	0.08 ± 0.22
	40	80	60 ± 11	0.13 ± 0.21
Diacetyl	25	5	57.9 ± 6.9	-0.08 ± 0.27
	25	80	55.6 ± 8.1	0.09 ± 0.34
	40	5	41.9 ± 3.3*	0.29 ± 0.16**
	40	80	53.9 ± 7.6	0.26 ± 0.23**
2,3-Pentanedione	25	5	52.2 ± 8.4	-0.04 ± 0.37
	25	80	50.0 ± 5.0	-0.04 ± 0.23
	40	5	40.7 ± 3.6*	0.24 ± 0.19**
	40	80	49.1 ± 5.0	0.05 ± 0.17

*: The slope of the linear regression equation was significantly different from other temperature and RH conditions at $p \leq 0.05$ for the same ketone.
**: The intercept of the linear regression equation was significantly different from zero at $p \leq 0.05$.

Table 4-19: Lowest Detected Ketone Vapor Concentrations with ≤ 10 % CVs at Different Conditions

Ketone	Temp (ºC)	RH (%)	8-hour TWA Conc. ± SD (ppb)
Acetoin	25	5	4.19 ± 0.25
	25	80	4.12 ± 0.17
	40	5	4.24 ± 0.10
	40	80	3.27 ± 0.28
Diacetyl	25	5	8.06 ± 0.56
	25	80	7.7 ± 0.55
	40	5	4.81 ± 0.19
	40	80	4.622 ± 0.015
2,3-Pentanedione	25	5	7.01 ± 0.29
	25	80	5.63 ± 0.23
	40	5	4.47 ± 0.30
	40	80	3.62 ± 0.36

4.8.8 Experimental Sampling Constants for Sampling Duration Effects

Figure 7-16, Figure 7-18, and Figure 7-20 in the Appendix demonstrate the experimental sampling constants at 25 ºC and 5 % RH for acetoin, diacetyl, and 2,3-pentanedione respectively by adding the target TLV-TWA for 8-hour exposure data to Figure 7-4, Figure 7-8, and Figure 7-12. Similarly, Figure 7-17, Figure 7-19, and Figure 7-21 in the Appendix demonstrate the experimental sampling constants at 25 ºC and 80 % RH for acetoin, diacetyl, and 2,3-pentanedione respectively by adding the 8-hour data to Figure 7-5, Figure 7-9, and Figure 7-13. Table 4-20 summarizes the ketone sampling constants from Figure 7-16 through Figure 7-21. All of the intercepts of the linear regression in Table 4-20 had no significant difference from zero at $p \leq 0.05$ for either acetoin, diacetyl, or 2,3-pentanedione. Also, each sampling constant at the specific temperature and RH in Table 4-18 and Table 4-20 was compared using a Student t-test and equation 3.8. All of the acetoin, diacetyl, and 2,3-pentanedione sampling constants with and without 8-hour samples had no statistical difference. In fact, the measurements from the 8-hour samples were superimposable onto the linear regression line of 1-hour samples for each ketone.

Table 4-20: Summary of Regression Equation to Determine Dependence on Sampling Duration at 25 °C and Different RHs

Ketone	Temp. (ºC)	RH (%)	Slope ± SD (mL/min)	Intercept ± SD (µg)
Acetoin	25	5	58.5 ± 7.7	0.10 ± 0.21
		80	60.5 ± 8.9	0.14 ± 0.23
Diacetyl	25	5	56.3 ± 7.2	0.00 ± 0.26
		80	54.4 ± 7.7	0.15 ± 0.29
2,3-Pentanedione	25	5	51.8 ± 7.4	-0.02 ± 0.30
		80	48.7 ± 5.0	0.04 ± 0.21

4.8.9 Sampling Capacity

Figure 7-22 in the Appendix demonstrates the pellet molar capacity for acetoin when acetoin vapor was sampled alone at 80 % RH and 25 ºC at various concentrations and durations. In Figure 7-22, the total moles of acetoin sampled over sampling time (µmol) (Y axis) were plotted against the true acetoin air concentration (µmol/mL) × sampling time (min) (X axis). Moles were used instead of mass in the capacity study because it was more convenient for comparing capacity among different ketones. The molar capacity of the pellet was determined where the moles collected on the pellet no longer increased as the concentration × sampling time increased. The pellet molar capacity for acetoin was about 45 µmol.

Figure 7-23 and Figure 7-24 in the Appendix demonstrate the pellet molar capacity for diacetyl and 2,3-pentanedione respectively when their ketone vapor alone was sampled at 80 % RH and 25 ºC at various concentrations and durations. The pellet molar capacities for diacetyl and 2,3-pentanedione were about 20 and 24 µmol respectively. Thus, acetoin was collected on the pellet to the greatest extent followed by 2,3-pentanedione and diacetyl.

Figure 7-25 through Figure 7-27 in the Appendix demonstrate the pellet molar capacity for acetoin, diacetyl, and 2,3-pentanedione respectively when ketone vapor mixtures were sampled at 80 % RH and 25 ºC at various concentrations and durations. The same molar concentration was generated for each ketone simultaneously in the exposure chamber to compare the pellet molar capacities. The pellet molar capacities for acetoin, diacetyl, and 2,3-pentanedione were about 30, 14, and 18 µmol respectively. Although each ketone capacity as a mixture decreased compared to when ketones were generated individually, the order of greatest capacity remained the same.

4.8.10 Determination of Passive Sampler Storage Periods after Sampling

Table 7-36 in the Appendix indicates the acetoin sample recoveries when a vapor mixture at 80 ppb at 25 ºC and 5 % RH was sampled for 1 hour, and the pellets were stored at different temperature and duration combinations. The recoveries were calculated based on the experimental sampling constant (59 mL/min) determined from 1-hour sampling at 25 ºC and 5 % RH in Figure 7-4 in the Appendix. The p-values indicate whether the recoveries were statistically different from the first analysis (0 day storage). All the samples maintained statistically the same recoveries at all the tested storage temperature and duration combinations. Furthermore, all of the mean recoveries were within 75-125 % range.

Table 7-36 and Table 7-37 in the Appendix indicate the diacetyl and 2,3-pentanedione sample recoveries respectively when vapor mixture at 80 ppb at 25 ºC and 5 % RH was sampled for 1 hour, and the pellets were stored at different temperature and duration combinations. The recoveries for diacetyl and 2,3-pentanedione were calculated based on the experimental sampling constants (58 and 52 mL/min respectively) determined in Figure 7-8 and Figure 7-12 in the Appendix respectively. All the samples maintained statistically the same recoveries at all the tested storage temperature and duration combinations for diacetyl. However, the recoveries were statistically different for 2,3-pentanedione except when samples were stored at -20 ºC for 30 days. However, all of the mean recoveries were above 75 % and below 125 % for both diacetyl and 2,3-pentanedione.

5 Discussion

This section summarizes the critical findings from the results. The results are also compared with those from other published air sampling methods for acetoin, diacetyl, and 2,3-pentanedione.

5.1 Synthesis of PFBHA O-Oximes

As shown in Table 4-1, the results of the PFBHA O-oxime synthesis within one hour reaction time in aqueous solution indicated that acetoin was quantifiable via its mono-derivative; whereas, diacetyl and 2,3-pentanedione were quantifiable by summing their mono- and di-derivatives. It took longer for 2,3-pentanedione to become its di-derivative compared to diacetyl possibly because 2,3-pentanedione was more sterically hindered and more non-polar than diacetyl.

5.2 Development of GC-MS Analytical Methods

It was necessary to select M-heptanal as an internal standard relative to DBP and utilize SIM to achieve sensitivity. Since M-heptanal was analyzable using m/z 181 common with M-diacetyl and D-diacetyl, the AUC ratio of M- or D- diacetyl/M-heptanal remained the same even if the GC-MS conditions changed over time. Because DBP required the use of m/z 121 which differed from m/z 181 for the target O-oxime, the AUC ratio did not stay the same throughout the day due to changes in GC-MS conditions. Another possibility why the AUC ratio was stable using M-heptanal as an internal standard compared to DBP was because the volatility of M-heptanal, M-diacetyl, and D-diacetyl was probably similar compared to DBP.

Furthermore, the analysis method required summation of E- and Z- isomers. If the ratio of isomers at each condition remained the same each time, it was possible to use the largest

isomer peak to simplify the analysis. However, the ratio changed depending on how and where diacetyl became D-diacetyl as shown in Table 7-1. For example, some diacetyl became D-diacetyl inside of the sampling tube during vapor spiking, but some also became D-diacetyl during the desorption step. Also, the longer the storage period after vapor spiking, the more diacetyl became D-diacetyl inside of the tube. Thus, the complexity of the reaction made it harder to achieve consistent isomer ratios. As concentration became lower, the isomer ratio changed because the largest peak of the isomers had a lower LQL than smaller peaks, and the smaller peaks became indistinguishable from the background.

By changing the GC temperature program, it is possible to combine the isomers into one representative peak. However, since retention times of D-diacetyl isomers and D-2,3-pentanedione isomers overlapped as shown in Table 4-3, it was necessary to separate each isomer to quantify the mixture of ketones. However, OSHA Method 1012[17] used only one D-diacetyl peak to quantify the concentration of diacetyl, and there was no information about the E- and Z- isomers. If the temperature program was developed where the E- and Z- isomer peaks come together, there may be interference with D-2,3-pentanedione peaks when 2,3-pentanedione is present as described in Section 4.4. Also, M-acetoin peaks may interfere with M-2,3-pentanedione if all 2,3-pentanedione does not turn into D-2,3-pentanedione within 36 hours of reaction time. Thus, OSHA Method 1012 may have potential disadvantages when diacetyl and acetoin are sampled in the presence of 2,3-pentanedione. The method developed through this research overcomes these potential disadvantages by using m/z 240 in SIM as described in Section 4.5 with the optimized temperature program. GC-MS, however, is more expensive than GC-ECD.

5.3 Liquid Spiking onto Dynamic Sampling Tubes

The recovery for diacetyl and 2,3-pentanedione liquid/solid spiking with the Tenax-water-hexane method was higher than with the Tenax-hexane method as shown in Table 4-9 because unreacted ketones which physically adsorbed on the solid sorbent surface were able to react better with PFBHA once the solid sorbents were suspended in aqueous solution before the hexane extraction. This may have been because the ketone may have physically adsorbed with only partial chemisorption due to steric hindrance. Overtime, the chemisorption process would continue, and the water step hastened the derivatization.

Since di-substituted O-oximes had lower LQLs than mono-substituted O-oximes, it would be ideal if the original ketone concentrations were calculated using only di-substituted O-oximes for diacetyl and 2,3-pentanedione. Analyzing only the di-derivative instead of summing mono- and di-derivatives also could make analysis simpler. However, as shown in Table 4-9, even with a desorption/reaction time of 3 days in aqueous solution, there was M-diacetyl and M-2,3-pentanedione left in the solution, even though diacetyl turned into D-diacetyl in aqueous solution in 2 days as mentioned in Section 4.1. The major difference was the presence of Tenax TA in the air sampling procedure. The major problem with summing mono- and di-derivatives occurs at low concentrations when the mono-derivative concentrations are below their LQLs.

5.4 Vapor Sampling using Dynamic Sampling Tubes

The most important results from the dynamic sampling study using the PFBHA coated Tenax tubes were the independence of temperature, RH and sampling duration, and mixture effects with minor exceptions as noted below. Table 5-1 summarizes the percent mean recoveries with SD under the different tested conditions using the 200 mg coated Tenax TA solid

sorbent tubes and the Tenax-water-hexane method for analysis. The recoveries shown in Table 5-1 were from studies conducted using the three ketones in a mixture unless noted otherwise. For vapor spiking, a flow rate of 100 mL/min was used for all the studies, this previously being shown to not cause breakthrough at these vapor concentrations.

Vapor sampling recoveries for acetoin, diacetyl, and 2,3-pentanedione for dynamic sampling were acceptable for most of the studied conditions because the CVs were less than 10 % and the percent relative errors were less than 25 % as required by NIOSH criteria.[148] Thus, low and high RHs (5 and 80 %) and different temperatures (5, 25, and 40 °C) did not influence the sampling recoveries except at 1 ppb where sensitivity became a problem and at 5 °C for acetoin where it solidified since its melting point is 15 °C.[19] Also, acetoin vapor will adsorb more on the gas bag inner walls at 5 ºC. After 48 L out of 60 L were sampled at 5 ºC, the gas bag was heated with a hairdryer and placed in the environmental chamber at 25 ºC. When the remaining air was sampled, the acetoin recovery was over 125 %. This phenomenon was probably because the adsorbed acetoin was vaporized due to heating. Thus, the static method with Tedlar gas bags was not suitable to determine the sampling efficiency of acetoin at 5 °C.

Among the three ketones, the sampling tube had higher capacity for acetoin and 2,3-pentanedione compared to diacetyl as demonstrated in Figure 4-7 through Figure 4-9. As explained in Section 4.7.10, the sampling tube was estimated to collect ketone vapor with approximately 75 % recovery at 8.2, 2.7, and 11 ppm for acetoin, diacetyl, and 2,3-pentanedione respectively at 100 mL/min for 8-hour sampling. The result was as expected for acetoin since acetoin had only one carbonyl group to react with PFBHA; whereas, diacetyl and 2,3-pentanedione had two carbonyl groups to react with PFBHA. The capacity of the sampling tube was higher for 2,3-pentanedione than diacetyl. This phenomenon could be explained if 2,3-

pentanedione was acting like a mono-ketone instead of di-ketone in terms of reaction with PFBHA. Since it took longer for 2,3-pentanedione to react in aqueous solution than diacetyl, the second carbonyl group of 2,3-pentanedione might be much slower to react with PFBHA compared to diacetyl during vapor sampling at higher concentrations at high flow rates. Thus, 2,3-pentanedione mostly remained as M-2,3-pentanedione in the tube and acted like a mono-ketone instead of a di-ketone. Also, 2,3-pentanedione might physically adsorb more than diacetyl on Tenax TA.

When the capacity of the tube was determined using the ketone mixture vapor, the comparison of mixture recovery at 5 and 80 % RH indicated that the capacity of the solid sorbent tubes is higher with higher RH. This could be because since ketones react faster with PFBHA in aqueous solution and there may be water condensation in the micropores. Therefore, the water inside of the tubes at high RH could be enhancing the reaction of ketones and PFBHA during sampling.

Overall, the capacity of the sampling tube was much higher (about 270 times higher) than the 2012 ACGIH TLV-TWA equivalent for diacetyl. Thus, the 200 mg PFBHA coated Tenax TA dynamic sampling tubes are practical for use in the field without being as bulky as the 600 mg silica gel tubes in series required for OSHA Method 1012, 1013, and 1016.

Overall acetoin, diacetyl, and 2,3-pentanedione vapor recoveries were 90.2 ± 6.9, 92.2 ± 5.9, and 82.5 ± 4.4 % respectively with CVs of 7.7, 6.4, and 5.3 respectively when the data (vapor sampling recoveries at 5, 10, and 20 ppb, 25 °C, both 5 and 80 % RH, and 100 mL/min for 8 hours) were pooled in Table 4-11. The vapor sampling recoveries included ketone and PFBHA reaction efficiency, PFBHA O-oxime desorption efficiency, and any gas bag wall

adsorption effects. The tubes could be stored at room temperature for at least 30 days, the data not being different at -20 °C.

Table 5-1: Comparison of Recoveries (%) at Different Conditions

		Acetoin	Diacetyl	2,3-Pentanedione
Desorption Efficiency	PFBHA O-Oxime Liquid Spiking[A]	96.0 ± 5.0	98.2 ± 4.2	97.2 ± 3.1
Reaction/Desorption Efficiency	Ketone Liquid Spiking[A]	92.4 ± 8.3	94.6 ± 9.0	92.5 ± 8.1
Individual vs. Mixture	Individual[B]	100.8 ± 2.6	94.1 ± 2.4	97.3 ± 4.6
	Mixture[B]	93.2 ± 8.0	87.7 ± 1.3	93.1 ± 1.8
Duration Test	1 hr[C]	93.2 ± 8.0	87.7 ± 1.3	93.1 ± 1.8
	8 hrs[D]	98.3 ± 9.2	93.7 ± 6.3	80.5 ± 2.9
RH Test	5 % RH[E]	94.1 ± 6.4	89.6 ± 6.6	82.1 ± 5.0
	80 % RH[E]	86.3 ± 5.1	94.8 ± 3.8	82.9 ± 3.9
	5 and 80 % RH[E]	90.2 ± 6.9	92.2 ± 5.9	82.5 ± 4.4
Temperature Test at 5 % RH	5 ºC[F]	55.9 ± 3.3	90.8 ± 2.6	81.8 ± 1.4
	25 ºC[F]	98.3 ± 9.2	93.7 ± 6.3	80.5 ± 2.9
	40 ºC[F]	85.4 ± 7.9	93.2 ± 4.3	77.9 ± 3.1
Temperature Test at 80 % RH	5 ºC[F]	63.1 ± 6.0	86.96 ± 0.74	78.5 ± 4.5
	25 ºC[F]	85.1 ± 4.8	94.4 ± 2.3	86.3 ± 3.4
	40 ºC[F]	98.4 ± 9.5	102.6 ± 2.5	88.3 ± 3.3

A: Tested 8-hour TWA equivalent concentration: 1-20 ppb
B: Tested at 80 ppb, 25 ºC and 5 % RH for 1 hr
C: Tested at 80 ppb, 25 ºC and 5 % RH
D: Tested at 10 ppb, 25 ºC and 5 % RH
E: Tested at 5, 10, and 20 ppb and 25 ºC for 8 hrs
F: Tested at 20 ppb for 8 hrs

5.5 Liquid Spiking onto Passive Sampling Pellets

The PFBHA O-oxime liquid/solid spiking recoveries indicate that the O-oxime derivatives were desorbed from the pellet with high efficiencies as the mean recoveries were 86.9 ± 4.1, 85.5 ± 2.9, and 95.3 ± 5.1 % for M-acetoin, D-diacetyl, and D-2,3-pentanedione respectively. The results also indicated that solid sorbents were transferred into a centrifuge tube without measurable loss even though the pellets had to be broken. The pellet was compressed

with 6 tons of pressure, yet was able to regain powdered form after breaking the pellet with a spatula.

Although the ketone liquid/solid spiking recoveries exceeded 75 % for all ketones, acetoin and 2,3-pentanedione recoveries were statistically lower compared to PFBHA O-oxime liquid/solid spiking. These lower recoveries might be due to evaporation and desorption of ketones from the pellet surface. If ketones reacted instantly with PFBHA in the pellets and formed O-oximes, the ketone liquid/solid spiking recoveries should be equivalent to those from PFBHA O-oxime liquid/solid spiking. However, the majority of ketones reacted with PFBHA on the pellets (chemisorption) or they remained physically adsorbed onto the pellets and reacted once the solid sorbents were suspended in water. The persistence of physical adsorption may increase chances of escape of adsorbed vapors that would not occur after complete chemisorption.

5.6 Experimental Sampling Constants of Passive Sampler

The most important results from the passive sampling study at 25 ºC using the custom developed samplers were the independence of RH and sampling duration effects. The results from the sampling duration study confirm that 1-hour exposure experiments provided equivalent data to 8-hour exposure experiments using the custom developed pellets. The one hour experiments minimized safety and time problems during the research. Since the sampling constants at 25 ºC for acetoin, diacetyl, and 2,3-pentanedione showed no statistical difference at different RH and sampling durations, all of the data were pooled for each ketone. Figure 5-1 through Figure 5-3 demonstrate the experimental sampling constants for acetoin, diacetyl, and

2,3-pentanedione respectively when all of the data were pooled. The intercepts of the linear regression equation had no significant difference from zero at $p \leq 0.05$ except for acetoin.

Overall experimental sampling constants for acetoin, diacetyl, and 2,3-pentanedione at 25 ºC were determined as 59.4 ± 8.5, 55.3 ± 7.6, and 50.0 ± 6.3 mL/min respectively. Table 5-2 compares the theoretical and experimental sampling constants at 25 and 40 ºC. Since the theoretical sampling constants for acetoin, diacetyl, and 2,3-pentanedione at 25 ºC were 114, 116, and 105 mL/min respectively, the experimental sampling constants were about half of the expected values. The lower experimental sampling constants were probably due to lower desorption and reaction efficiencies because the experimental sampling constants were determined using raw data for the ketones extracted from the pellet. The experimental sampling constant likely underestimated the actual sampling constant. However, by using the identified experimental sampling constant, the true vapor concentration is determined without requiring correction for reaction and desorption efficiencies.

Although there was independence of RH at 25 ºC, there were RH effects at 40 ºC. Since the experimental sampling constants were 51.3 ± 5.8, 41.9 ± 3.3, and 40.7 ± 3.6 mL/min at 40 ºC and 5 % RH and 60 ± 11, 53.9 ± 7.6, and 49.1 ± 5.0 mL/min at 40 ºC and 80 % RH for acetoin, diacetyl, and 2,3-pentanedione respectively, the experimental sampling constants were statistically significantly higher at higher RH at constant temperature of 40 ºC. Since ketones react well with PFBHA in aqueous solution, ketones adsorbed on the pellet might have reacted faster at 80 % RH compared to 5 % RH. However, since this phenomenon occurred only at 40 ºC, the mechanism is unknown. The 25 ºC data showed no effects of temperature, RH, or exposure duration as previously observed for aldehydes[137, 140] and unsterically hindered ketones.[141]

Although there was independence of temperature at 80 % RH, there were temperature effects at 5 % RH. Since the experimental sampling constants were 58.8 ± 8.0, 57.9 ± 6.9, and 52.2 ± 8.4 mL/min at 25 ºC and 5 % RH and 51.3 ± 5.8, 41.9 ± 3.3, and 40.7 ± 3.6 mL/min at 40 ºC and 5 % RH for acetoin, diacetyl, and 2,3-pentanedione respectively, the experimental sampling constants were statistically lower at higher temperature at constant RH of 5 %. Since the theoretical sampling constants are 114, 116, and 105 mL/min at 25 ºC and 124, 127, and 115 at 40 ºC, the sampling constants are expected to be higher at higher temperature. Thus, the temperature effect results from this experiment contradicted theory. When RH was kept at 5 % for both 25 ºC and 40 ºC, the absolute humidity was 2.2 times higher at 40 ºC. However, since the RH of 5 % was really low, no condensation would have occurred in the sampler. Thus, the experimental sampling constants were lower at higher temperature for unknown reasons.

Since the sampling constants at 40 ºC and 80 % RH were not statistically different from 25 ºC with 5 or 80 % RH, all of the data were pooled for each ketone as shown in Figure 5-4 through Figure 5-6. Overall experimental sampling constants for acetoin, diacetyl, and 2,3-pentanedione at 25 ºC were determined as 59.4 ± 8.7, 54.8 ± 8.2, and 49.8 ± 5.8 mL/min respectively. The intercepts of the linear regression equation had no significant difference from zero at $p \leq 0.05$ for 2,3-pentanedione, but showed significant differences for acetoin and diacetyl.

Since the intercept was not zero for acetoin and diacetyl, the regression lines both with and without the intercept were used to calculate the mass collected on the pellet at 10 ppb 8-hour TWA. If 10 ppb of acetoin, diacetyl, and 2,3-pentanedione were sampled at 25 ºC for 8 hours using the custom developed passive sampler and only the slope (experimental sampling constant) was used to calculate the mass collected on the pellet without using the intercept, there would be -11, -13, and -2.6 % relative errors respectively compared to using the slope with intercept to

calculate. Since acetoin and diacetyl gave more than 10 % error without using the intercept, it is best to use both slope and intercept to determine their concentrations.

From the capacity study, the pellet molar capacities for acetoin, diacetyl, and 2,3-pentanedione were determined as about 45, 20, and 24 μmol respectively when no other aldehydes and ketones were present. Thus, acetoin was collected on the pellet to the greatest extent followed by 2,3-pentanedione and diacetyl. The highest capacity was expected for acetoin since acetoin had only one carbonyl group to react with PFBHA; whereas, diacetyl and 2,3-pentanedione had two carbonyl groups to react with PFBHA.

Another important finding from the capacity study was that the sampling constants decreased as concentration and sampling time increased as shown in Figure 7-22 through Figure 7-27. This phenomenon is explained by the Langmuir adsorption isotherm equation. Although a sampling constant could be determined within a small concentration and sampling time range, the sampling constant could not be applied to higher concentrations.

The pellet molar capacities for acetoin, diacetyl, and 2,3-pentanedione were about 30, 14, and 18 μmol respectively when the three ketones existed together at the same concentration. Although each ketone capacity as a mixture decreased compared to when ketones were generated individually, the order of greatest capacity remained the same. Thus, a greater capacity allows for reliable sampling in the presence of other aldehydes and ketones which may likely be present in the field. Since the capacity of the 300 mg PFBHA coated Tenax TA pellet was much higher than the 2012 ACGIH TLV-TWA for diacetyl, the custom developed passive samplers are practical for use in the field.

Table 5-2: Comparison of Theoretical and Experimental Sampling Constants at Different Temperatures and RHs

Ketone	Temp. (ºC)	Sampling Constant ± SD (mL/min)		
		Theoretical	Experimental at 5 % RH	Experimental at 80 % RH
Acetoin	25	114	58.8 ± 8.0	60.4 ± 9.4
	40	124	51.3 ± 5.8	60 ± 11
Diacetyl	25	116	57.9 ± 6.9	55.6 ± 8.1
	40	127	41.9 ± 3.3	53.9 ± 7.6
2,3-Pentanedione	25	105	52.2 ± 8.4	50.0 ± 5.0
	40	115	40.7 ± 3.6	49.1 ± 5.0

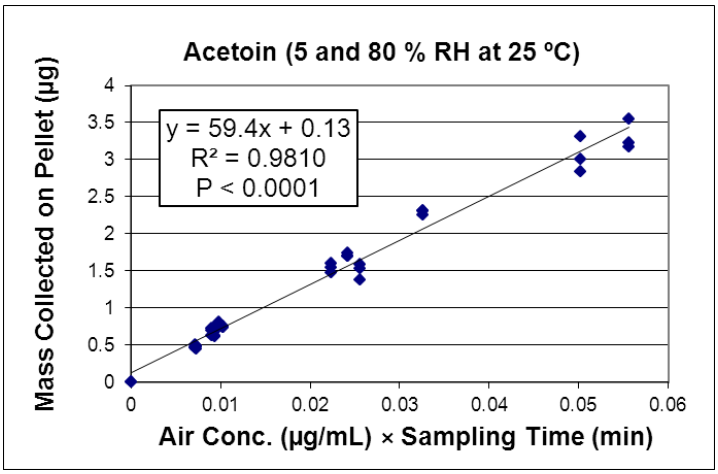


Figure 5-1: Acetoin Experimental Sampling Constant from Pooled Data at 25 ºC

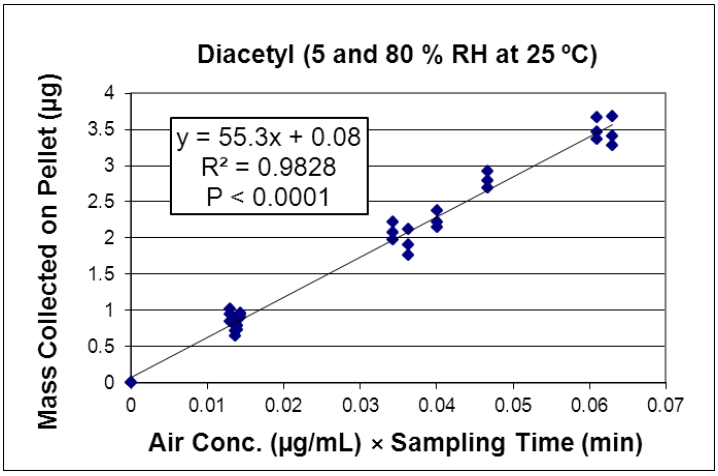


Figure 5-2: Diacetyl Experimental Sampling Constant from Pooled Data at 25 ºC

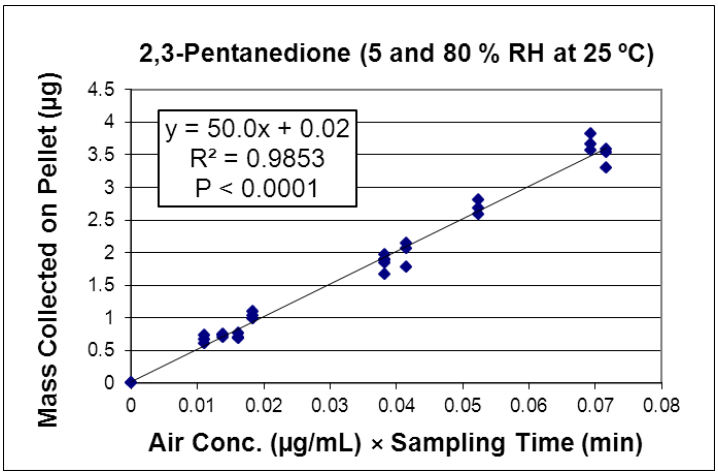


Figure 5-3: 2,3-Pentanedione Experimental Sampling Constant from Pooled Data at 25 ºC

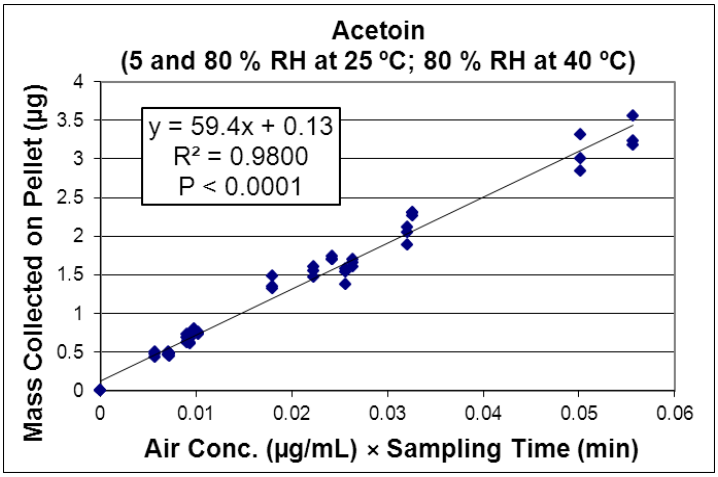


Figure 5-4: Acetoin Experimental Sampling Constant from Pooled Data at 25 ºC with 5 and 80 % RH and 40 ºC with 80 % RH

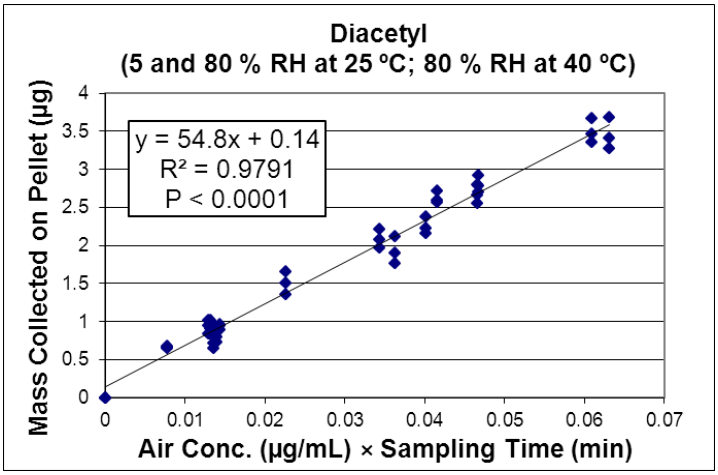


Figure 5-5: Diacetyl Experimental Sampling Constant from Pooled Data at 25 ºC with 5 and 80 % RH and 40 ºC with 80 % RH

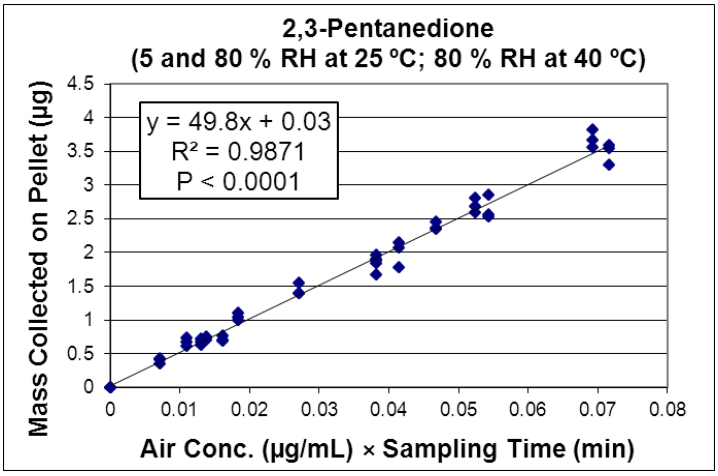


Figure 5-6: 2,3-Pentanedione Experimental Sampling Constant from Pooled Data at 25 ºC with 5 and 80 % RH and 40 ºC with 80 % RH

5.7 Method Sensitivity and Selectivity

The most sensitive published method for acetoin and diacetyl is OSHA Method 1012[17] as mentioned in Section 2.7.2. The RQLs for acetoin and diacetyl are 1.5 ppb and 1.3 ppb respectively. The recoveries of acetoin and diacetyl were above 98.4 and 98.0 % respectively with the overall procedure precision of 9.9 and 10 % respectively from the OSHA storage study. The most sensitive published method for 2,3-pentanedione is OSHA 1016[23] as mentioned in Section 2.7.2. The RQL for 2,3-pentanedione is 9.3 ppb. The recovery of 2,3-pentanedione was above 91.3 % with the overall procedure precision of 10 % from the OSHA storage study. Furthermore, the lowest working ranges were 170 ppb for acetoin using NIOSH Method 2558[16] and 57 ppb for diacetyl using NIOSH Method 2557.[115] The RQLs were 11 and 12 ppb for acetoin and diacetyl respectively using OSHA Method 1013,[18] and the RQL was 280 ppb for diacetyl using OSHA Method PV2118.[117]

The vapor sampling LQLs for the ketones were between 1 to 5 ppb 8-hour TWA using the PFBHA coated Tenax TA method for both dynamic and passive samplers. The sensitivity

below 5 ppb is dependent on the GC-MS sensitivity on the specific day, and on the manual integration technique used to obtain AUCs. Although OSHA Method 1012 provides about the same sensitivity for acetoin and diacetyl compared with this PFBHA coated Tenax TA dynamic and passive sampling methods, the Tenax TA method is definitely more sensitive for 2,3-pentanedione compared to OSHA Method 1016. Overall, the PFBHA coated Tenax TA dynamic and passive sampling methods provided more sensitivity compared to OSHA Method 1013 and PV2118 as well as NIOSH Method 2557 and 2558.

The PFBHA Tenax TA method could be more sensitive if the desorption duration was extended until all the M-diacetyl and M-2,3-pentanedione became D-diacetyl and D-2,3-pentanedione because di-substituted derivatives have higher response factors than the mono-substituted derivatives. Also, each ketone would become one di-substituted derivative instead of two derivatives. However, in the presence of Tenax TA, the reaction does not go to completion in the diketone water reaction stage of the desorption. This is an area for future research.

Furthermore, there were lower background peaks around the retention time of D-diacetyl compared to M-diacetyl. The limitation of the sensitivity was largely due to the impurity PFBHA peaks in the method blank. The sensitivity would increase if the baseline became lower for M-diacetyl quantitation.

Acetoin vapor sampling sensitivity will increase if no M-2,3-pentanedione is present or if the air does not contain 2,3-pentanedione. If there were no peaks around the retention time of acetoin, m/z 181 could be used to quantify acetoin instead of m/z 240 which is less sensitive by approximately a factor of 2.

This Tenax TA method was developed to provide enough sensitivity for the 2012 ACGIH TLV-TWA for diacetyl, so a relatively high flow rate (100 mL/min) was used. Due to the high

flow rate, this method is not recommended for higher concentrations (> 8.2, 2.7, and 11 ppm for acetoin, diacetyl, and 2,3-pentanedione respectively for 8-hour TWA) where breakthrough occurred. In such cases, a backup tube is recommended. However, when sampling is required at higher concentration, a lower flow rate, such as 10 mL/min and a shorter sampling time can be used to minimize breakthrough, as was also done during this research. A lower flow rate may allow more efficient reaction for less reactive ketones like diacetyl and 2,3-pentanedione, but it has the disadvantage of less sensitivity. The developed method may be more selective than the OSHA Method 1012 for air mixtures since D-diacetyl may interfere with D-2,3-pentanedione. The developed MS method resolves the chromatograph interference through the use of SIM, especially m/z 240 in addition to m/z 181.

5.8 Cost and Benefit

To develop practical air sampling and analytical methods, it is important to understand the costs and benefits. For both dynamic and passive sampling, effort is required to prepare tubes and pellets for sampling since they are not commercially available. For instance, Tenax TA has to be initially cleaned and then coated by PFBHA; solid sorbents must be manually packed into tubes or formed into pellets.

For dynamic sampling, pumps must be calibrated and operated at a specific flow rate for successful sampling. Passive sampling is more practical and cost effective since no pump or bulky equipment is required. However, the sampling constant must be known along with its air concentration validity range. Several important criteria for feasible industrial hygiene analytical methods involve the design of personal monitoring samplers with compact size, weight, and convenience.[52] Published OSHA and NIOSH methods are only for dynamic sampling which

require operation of pumps. However, the PFBHA coated Tenax TA dynamic tubes are less bulky compared to those used in the OSHA methods 1012, 1013, and 1016. The greatest benefits of both the dynamic and passive samplers are that they can detect below 10 ppb both accurately and precisely at various temperatures and RHs regardless of whether acetoin, diacetyl, and 2,3-pentanedione are in a vapor mixture. Since the 2012 ACGIH TLV-TWA for diacetyl is 10 ppb, the dynamic and passive samplers are practical for the field sampling in the personal breathing zone.

5.9 Conclusions

Overall, the hypothesis of this research was proven correct as quantitative, selective, and sensitive dynamic and passive air sampling methods were developed for acetoin, diacetyl, and 2,3-pentanedione simultaneously based on PFBHA and Tenax TA. The analytical method developed in this research allowed the successful identification and quantitation of the ketones. In fact, these methods are more sensitive than currently established methods including NIOSH Methods 2557[115] and 2558[16] as well as OSHA Methods 1013,[18] 1016,[23] and PV2118.[117] Furthermore, the overall precision for both dynamic and passive sampling methods was less than 10 % within confirmed air concentration range.

The dynamic sampling method can allow identification and quantitation of ketones in mixture with above 75 % recoveries independent of studied RH, sampling times, and temperatures except at 5 ºC for acetoin. Similarly, the passive sampler can allow identification of ketones in mixture, and the concentration of ketones can be determined accurately and precisely using the experimental sampling constant equation determined from this research at a given temperature and RH. The capacity of the dynamic and passive samplers was well above

the target concentration of 10 ppb specified in the ACGIH TLV-TWA for diacetyl. Even after storage periods of 30 days at room temperature, all of the ketones can be recovered above the 75 % NIOSH criteria. Both the dynamic and passive methods are promising and should be utilized in the field to measure the concentration of acetoin, diacetyl, and 2,3-pentanedione.

6 Suggestions for Future Research

One of the most important areas of future research involves decreasing the background of the PFBHA impurity peaks, which will lower the LQL for both the passive and dynamic sampling methods. Although the background level was small for M-acetoin, M-2,3-pentanedione, D-diacetyl, and D-2,3-pentanedione, there was a relatively large peak around the retention time for the M-diacetyl peaks. By decreasing the background peaks, diacetyl can be quantified at lower concentrations.

Another way to lower the LQL is to optimize the analytical methods where diacetyl and 2,3-pentanedione will complete reaction with PFBHA and become completely disubstituted. Even though M-diacetyl and M-2,3-pentanedione remained after 3 days of reaction time in aqueous solution in the presence of PFBHA coated Tenax TA, filtering Tenax TA may allow reaction completion. This will also involve possible losses and enhance the chance of contamination. Also, it may take 5 days to complete the reaction for 2,3-pentanedione even after filtering according to the synthesis study results mentioned in Section 4.1. A short and as timely method as possible was the preferred choice in this research.

Also, since air in the field may contain gases, vapors, and aerosols, the methods should be tested in the presence of particles and other volatile organic vapors, especially aldehydes and ketones that react with PFBHA. The Pyrex glass wool of the dynamic air sampling tube and the silicone membrane of the passive air sampler should prevent aerosol contamination so as to collect only gas and vapor. However, both the dynamic and passive sampler can collect other organic vapors, and other aldehydes and ketones will react with PFBHA. Thus, it is important to make sure that there are no interference peaks around the PFBHA O-oxime peaks used for

quantification during GC-MS analysis. If there are interference peaks, the temperature program should be modified by slowing the increase of the GC oven temperature at the retention times around the interference peaks.

The passive sampler should be tested at different face velocities since the face velocity of the sampler will change depending on the movement of the workers. Since the critical face velocity of the mini-passive sampler is 15-20 ft/min[140] and the range of face velocities encountered in a typical workplace is 20-30 ft/min,[141] the research for the custom passive samplers was performed at face velocities higher than 30 ft/min. That it, the critical face velocity of the mini-pellet was assumed for the custom developed pellet. However, it is important to determine the critical face velocity of the custom passive samplers to ensure the pellets can collect vapors at the determined sampling constant at different concentrations both accurately and precisely. The sensitivity of the passive sampler may also be increased at shorter path lengths. Furthermore, to be completely practical, a holder must be designed to keep the pellet stationary when worn by workers. The holder must ensure the diffusion path length between the pellet and the silicone membrane without detriment to the surface area of the pellet.

Although the NIOSH method development and evaluation suggestions include testing sets of 6 samplers each for concentrations of 0.1, 0.5, 1.0, and 2.0 times the exposure limit,[148] this research was not intended to follow the NIOSH validation plan, thus tests were performed only in triplicate. However, more samples may yield statistically sound data though this is unlikely given the data documented. Further analysis using the NIOSH validation plan can be performed such as testing six different humidity and temperature levels using triplicate samplers at three different flow rates for the dynamic sampler. A shortened validation protocol was used

in the present research that assumed if there was no statistical difference at the extreme conditions, there would be no statistical differences in between.

Even though field evaluation is not required in the NIOSH validation plan for the development and evaluation of methods,[148] both dynamic and passive samplers should be tested in the field. Since NIOSH has already visited the flavoring manufacturing and food production industries, such as popcorn plants, occupational exposure studies at movie theaters may be helpful to indicate concordance with OSHA methods. Area samples could be collected around the popcorn cooking area using both the dynamic and passive air sampling methods. Also, personal breathing zone samples could be collected by asking employees to wear the passive sampler and dynamic sampling tubes with pump while popping popcorn during their work shift. Furthermore, acetoin, diacetyl and 2,3-pentanedione concentrations in the microwave popcorn cooking headspace can be measured to identify consumer exposures using both the dynamic and passive samplers. The presence of aerosol would also need to be accounted for.

7 Appendix

Table 7-1: Isomer AUC Ratios of D-Diacetyl

Method	Isomer	Sample 1	Sample 2	Sample 3	Mean	SD	CV
Tenax-Water-Hexane Standard	Isomer 1	0.0211	0.0236	0.0199	0.0215	0.0019	8.8
	Isomer 2	0.0883	0.0853	0.0801	0.0846	0.0042	4.9
	Isomer 3	0.8906	0.8911	0.9000	0.8939	0.0053	0.59
O-Oxime Liquid/Solid Spiking	Isomer 1	0.0115	0.0117	0.0112	0.01144	0.00025	2.2
	Isomer 2	0.0637	0.0652	0.0678	0.0656	0.0020	3.1
	Isomer 3	0.9248	0.9231	0.9211	0.9230	0.0019	0.20
Ketone Liquid/Solid Spiking	Isomer 1	0.1082	0.1221	0.1064	0.1122	0.0086	7.6
	Isomer 2	0.2269	0.2376	0.2278	0.2308	0.0059	2.6
	Isomer 3	0.6649	0.6403	0.6657	0.657	0.014	2.2
Ketone Vapor/Solid Spiking	Isomer 1	0.1338	0.1296	0.1314	0.1316	0.0021	1.6
	Isomer 2	0.2256	0.2268	0.2289	0.2271	0.0017	0.74
	Isomer 3	0.6405	0.6436	0.6397	0.6413	0.0020	0.32

Table 7-2: SIM Ion Behavior of M-Acetoin for Pure Hexane Standard

M-Acetoin Mass Injected (ng)	5	10	25	50	75	100
Observed AUC 181+240	110537	575390	1337905	2218826	4497001	5568852
Extracted AUC 181	75443	404266	949730	1581517	3214858	4027248
Extracted AUC 240	35779	175251	397361	648457	1302160	1602586
Calculated AUC 181 + 240	111222	579517	1347091	2229974	4517018	5629834
% Extracted AUC 240	32.4	30.5	29.7	29.2	29.0	28.8
% Calc/Obs AUC 181+240	100.6	100.7	100.7	100.5	100.4	101.1

Table 7-3: SIM Ion Behavior of M-Acetoin for Tenax-Hexane Standard

M-Acetoin Mass Injected (ng)	5	10	25	50	75	100
Observed AUC 181+240	268594	639765	1510926	3163517	4193985	6115675
Extracted AUC 181	184134	456683	1089356	2256973	2996116	4372361
Extracted AUC 240	89728	193712	446847	925680	1214971	1751429
Calculated AUC 181 + 240	273862	650395	1536203	3182653	4211087	6123790
% Extracted AUC 240	33.4	30.3	29.6	29.3	29.0	28.6
% Calc/Obs AUC 181+240	102.0	101.7	101.7	100.6	100.4	100.1

Table 7-4: SIM Ion Behavior of M-Acetoin for Tenax-Water-Hexane Standard

M-Acetoin Mass Injected (ng)	5	10	25	50	75	100
Observed AUC 181+240	250007	624209	1520204	3216557	4222537	5532661
Extracted AUC 181	172331	436725	1078262	2290946	3020767	3969822
Extracted AUC 240	82470	194379	454356	944979	1228449	1607115
Calculated AUC 181 + 240	254801	631104	1532618	3235925	4249216	5576937
% Extracted AUC 240	33.0	31.1	29.9	29.4	29.1	29.0
% Calc/Obs AUC 181+240	101.9	101.1	100.8	100.6	100.6	100.8

Table 7-5: SIM Ion Behavior of M-Heptanal for Pure Hexane Standard

M-Heptanal Mass Injected (ng)	0	1	3	5	7	9
Observed AUC 181+239+240	0	109621	383666	682898	955826	1313177
Extracted AUC 181+240	0	89794	321064	557965	792120	1080370
Extracted AUC 239	0	19894	64850	117344	156353	231446
Calculated AUC 181+239+240	0	109688	385914	675309	948473	1311816
% Extracted AUC 239	N/A	18.1	16.9	17.2	16.4	17.6
% Calc/Obs AUC 181+239+240	N/A	100.1	100.6	98.9	99.2	99.9

Table 7-6: PFBHA O-Oxime Liquid/Solid Spiking Recoveries (%) with Tenax-Hexane Method over the 1-20 ppb Ketone Equivalent Range

PFBHA O-Oxime	Sample	Equivalent Concentration (ppb)			
		1	5	10	20
M-Acetoin	1	97.2	98.7	99.9	96.1
	2	99.3	90.0	92.4	97.3
	3	94.2	93.0	91.6	100.6
	Mean	96.9	93.9	94.6	98.0
	SD	2.6	4.4	4.6	2.3
	CV	2.7	4.7	4.8	2.4
D-Diacetyl	1	99.7	96.7	101.5	95.8
	2	96.2	90.1	91.9	97.2
	3	92.7	92.2	91.1	100.5
	Mean	96.2	93.0	94.8	97.8
	SD	3.5	3.4	5.8	2.4
	CV	3.6	3.6	6.1	2.4
D-2,3-Pentanedione	1	96.2	98.2	98.2	98.2
	2	94.3	91.0	91.0	91.0
	3	95.4	91.4	91.4	91.4
	Mean	95.3	93.6	93.6	93.6
	SD	1.0	4.1	4.1	4.1
	CV	1.0	4.3	4.3	4.3

Table 7-7: PFBHA O-Oxime Liquid/Solid Spiking Recoveries (%) with Tenax-Water-Hexane Method over the 1-20 ppb Ketone Equivalent Range

PFBHA O-Oxime	Sample	Equivalent Concentration (ppb)			
		1	5	10	20
M-Acetoin	1	107.8	93.23	101.9	97.1
	2	93.7	92.24	97.3	92.7
	3	99.3	93.96	91.3	91.7
	Mean	100.3	93.14	96.8	93.8
	SD	7.1	0.86	5.3	2.9
	CV	7.1	0.93	5.5	3.1
D-Diacetyl	1	107.3	99.2	102.6	98.1
	2	95.7	95.9	99.3	96.6
	3	99.8	96.2	98.2	90.0
	Mean	100.9	97.1	100.0	94.9
	SD	5.9	1.8	2.3	4.3
	CV	5.8	1.9	2.3	4.5
D-2,3-Pentanedione	1	98.3	100.0	101.3	97.9
	2	94.4	95.0	101.9	94.8
	3	98.4	94.0	98.8	92.1
	Mean	97.0	96.3	100.7	94.9
	SD	2.3	3.2	1.6	2.9
	CV	2.3	3.3	1.6	3.0

Table 7-8: Ketone Liquid/Solid Spiking Recoveries (%) with the Tenax-Hexane Method over the 1-20 ppb Ketone Equivalent Range

Ketone	Sample	Equivalent Concentration (ppb)			
		1	5	10	20
Acetoin	1	107.5	95.3	90.0	100.7
	2	110.2	93.8	92.9	86.4
	3	105.2	84.0	86.7	84.4
	Mean	107.6	91.0	89.9	90.5
	SD	2.5	6.1	3.1	8.9
	CV	2.3	6.7	3.4	9.8
Diacetyl	1	61.7	75.1	74.5	76.8
	2	70.8	74.2	74.4	68.8
	3	71.1	69.0	69.1	68.1
	Mean	67.9	72.8	72.7	71.2
	SD	5.3	3.3	3.1	4.8
	CV	7.8	4.5	4.3	6.8
2,3-Pentanedione	1	64.0	60.00	58.4	63.7
	2	65.0	61.20	58.8	58.1
	3	61.4	59.67	55.3	57.5
	Mean	63.5	60.29	57.5	59.8
	SD	1.8	0.81	1.9	3.4
	CV	2.9	1.3	3.3	5.7

Table 7-9: Ketone Liquid/Solid Spiking Recoveries (%) with Tenax-Water-Hexane Method over the 1-20 ppb Ketone Equivalent Range

Ketone	Sample	Equivalent Concentration (ppb)			
		1	5	10	20
Acetoin	1	87	98.5	87.2	85.7
	2	95	98.0	88.7	93.0
	3	114	91.9	82.9	86.1
	Mean	99	96.1	86.3	88.3
	SD	13	3.7	3.0	4.1
	CV	14	3.8	3.5	4.7
Diacetyl	1	88	102.7	86.6	91.3
	2	91	102.7	92.7	92.4
	3	118	88.0	92.9	89.8
	Mean	99	97.8	90.7	91.2
	SD	17	8.5	3.6	1.3
	CV	17	8.7	4.0	1.4
2,3-Pentanedione	1	84	104.2	81.1	93.9
	2	90	98.5	84.0	91.0
	3	106	97.8	94.7	85.5
	Mean	93	100.2	86.6	90.1
	SD	12	3.5	7.2	4.3
	CV	12	3.5	8.3	4.7

Table 7-10: Ketone Liquid/Liquid Spiking Recoveries (%) at 10 ppb Equivalent for the Tenax-Water-Hexane Method over 3 days

Ketone	Sample	3 days		
		Mono-	Di-	Total
Acetoin	1	90.6	N/A	90.6
	2	91.8	N/A	91.8
	3	94.7	N/A	94.7
	Mean	92.3	N/A	92.3
	SD	2.1	N/A	2.1
	CV	2.3	N/A	2.3
Diacetyl	1	21.4	77.97	99.3
	2	29.0	77.50	106.5
	3	22.1	79.25	101.3
	Mean	24.1	78.24	102.4
	SD	4.2	0.91	3.7
	CV	17	1.2	3.6
2,3-Pentanedione	1	31.0	68.9	99.9
	2	32.2	65.2	97.3
	3	23.1	65.8	89.0
	Mean	28.8	66.6	95.4
	SD	4.9	2.0	5.7
	CV	17	3.0	6.0

Table 7-11: Calibration of Thermometer

Alcohol Thermometer (Expected) (ºC)	Environmental Chamber Setting (ºC)	Multi-Function Thermometer (Observed) (ºC)
5	3.0	5.0
5	3.0	4.9
5	3.0	4.9
25	23.7	25.0
25	23.7	25.1
25	23.7	25.1
40	38.7	39.9
40	38.7	40.1
40	38.7	40.0

Table 7-12: Calibration of Hygrometer at 25 ºC and 760 mm Hg

	Expected RH (%)	Observed RH (%)	Error (%)
Dry Air	0.0	0.8	N/A
Lithium Chloride	11.3	11.3	0.0
Calcium Chloride	31.0	31.1	0.32
Magnesium Chloride	32.8	29.7	-9.4
Potassium Carbonate	43.2	39.9	-7.6
Sodium Dichromate	53.0	48.8	-7.9
Sodium Chloride	75.3	70.0	-7.0
Potassium Chloride	84.3	74.8	-11
Potassium Nitrate	93.6	82.1	-12

Table 7-13: Recovery (%) Comparison of Individual Ketone Vapors and Their Vapor Mixture

Ketone	Sample	80 ppb, 25 ºC, 5 % RH, 100 mL/min for 1 hour	
		Individual	Mixture
Acetoin	1	103.5	84.4
	2	100.9	95.0
	3	98.2	100.1
	Mean	100.8	93.2
	SD	2.6	8.0
	CV	2.6	8.6
	p-Value	0.2569	
Diacetyl	1	96.7	88.4
	2	93.6	86.2
	3	92.0	88.6
	Mean	94.1	87.7
	SD	2.4	1.3
	CV	2.6	1.5
	p-Value	0.0279*	
2,3-Pentanedione	1	101.4	91.3
	2	98.2	92.9
	3	92.3	95.0
	Mean	97.3	93.1
	SD	4.6	1.8
	CV	4.7	1.9
	p-Value	0.2361	

*: Significantly different at $p \leq 0.05$

Table 7-14: Recovery (%) Comparison of Vapor Sampling Time

Ketone	Sample	Mixture, 25 ºC, 5 % RH	
		80 ppb 100 mL/min for 1 hour	10 ppb 100 mL/min for 8 hours
Acetoin	1	84.4	108.8
	2	95.0	91.9
	3	100.1	94.0
	Mean	93.2	98.3
	SD	8.0	9.2
	CV	8.6	9.4
	p-Value	0.5108	
Diacetyl	1	88.4	87.1
	2	86.2	94.3
	3	88.6	99.7
	Mean	87.7	93.7
	SD	1.3	6.3
	CV	1.5	6.8
	p-Value	0.2506	
2,3-Pentanedione	1	91.3	83.6
	2	92.9	77.9
	3	95.0	79.9
	Mean	93.1	80.5
	SD	1.8	2.9
	CV	1.9	3.6
	p-Value	0.0078*	

*: Significantly different at $p \leq 0.05$

Table 7-15: Comparison of Dependence of Recoveries (%) on RH at 1 ppb

Ketone	Sample	Mixture, 1 ppb, 25 ºC, 100 mL/min for 8 hours	
		5 % RH	80 % RH
Acetoin	1	155	102.9
	2	155	98.0
	3	275	91.9
	Mean	195	97.6
	SD	69	5.5
	CV	36	5.7
	p-Value	0.1371	
Diacetyl	1	121	128
	2	181	119
	3	187	141
	Mean	163	129
	SD	36	11
	CV	22	8.7
	p-Value	0.2621	
2,3-Pentanedione	1	111.8	93
	2	102.2	90
	3	99.7	116
	Mean	104.5	100
	SD	6.4	14
	CV	6.1	14
	p-Value	0.6263	

Table 7-16: Comparison of Dependence of Recoveries (%) on RH at 5 ppb

Ketone	Sample	Mixture, 5 ppb, 25 ºC, 100 mL/min for 8 hours	
		5 % RH	80 % RH
Acetoin	1	90.5	89.1
	2	88.3	85.8
	3	89.8	86.1
	Mean	89.6	87.0
	SD	1.1	1.8
	CV	1.2	2.1
	p-Value	0.1327	
Diacetyl	1	81.2	97.5
	2	81.5	93.1
	3	86.1	87.7
	Mean	82.9	92.7
	SD	2.8	4.9
	CV	3.3	5.3
	p-Value	0.0569	
2,3-Pentanedione	1	76.3	78.7
	2	78.9	77.1
	3	82.9	80.0
	Mean	79.4	78.6
	SD	3.3	1.4
	CV	4.2	1.8
	p-Value	0.7358	

Table 7-17: Comparison of Dependence of Recoveries (%) on RH at 10 ppb

Ketone	Sample	Mixture, 10 ppb, 25 ºC, 100 mL/min for 8 hours	
		5 % RH	80 % RH
Acetoin	1	108.8	83.3
	2	91.9	90.5
	3	94.0	81.4
	Mean	98.3	85.1
	SD	9.2	4.8
	CV	9.4	5.6
	p-Value	0.1147	
Diacetyl	1	87.1	93.1
	2	94.3	93.1
	3	99.7	97.1
	Mean	93.7	94.4
	SD	6.3	2.3
	CV	6.8	2.5
	p-Value	0.8609	
2,3-Pentanedione	1	83.6	89.3
	2	77.9	86.8
	3	79.9	82.6
	Mean	80.5	86.3
	SD	2.9	3.4
	CV	3.6	3.9
	p-Value	0.0871	

Table 7-18: Comparison of Dependence of Recoveries (%) on RH at 20 ppb

Ketone	Sample	Mixture, 20 ppb, 25 ºC, 100 mL/min for 8 hours	
		5 % RH	80 % RH
Acetoin	1	99.9	80.1
	2	90.8	83.7
	3	92.9	96.4
	Mean	94.5	86.7
	SD	4.7	8.6
	CV	5.0	9.9
	p-Value	0.2630	
Diacetyl	1	89.6	93.5
	2	88.9	98.1
	3	97.7	100.4
	Mean	92.1	97.3
	SD	4.9	3.6
	CV	5.3	3.6
	p-Value	0.2063	
2,3-Pentanedione	1	88.8	82.7
	2	79.7	83.6
	3	91.0	85.6
	Mean	86.5	83.9
	SD	6.0	1.5
	CV	7.0	1.8
	p-Value	0.5499	

Table 7-19: Recovery (%) Dependence on Temperature during Sampling at 5 % RH

Ketone	Sample	Mixture, 20 ppb, 5 % RH, 100 mL/min for 8 hours		
		5 ºC	25 ºC	40 ºC
Acetoin	1	56.2	108.8	81.0
	2	52.4	91.9	80.8
	3	58.9	94.0	94.5
	Mean	55.9	98.3	85.4
	SD	3.3	9.2	7.9
	CV	5.8	9.4	9.2
Diacetyl	1	93.3	87.1	92.7
	2	88.2	94.3	89.1
	3	91.0	99.7	97.7
	Mean	90.8	93.7	93.2
	SD	2.6	6.3	4.3
	CV	2.8	6.8	4.6
2,3-Pentanedione	1	83.0	83.6	81.1
	2	82.2	77.9	75.0
	3	80.2	79.9	77.5
	Mean	81.8	80.5	77.9
	SD	1.4	2.9	3.1
	CV	1.8	3.6	3.9

Table 7-20: Recovery (%) Dependence on Temperature during Sampling at 80 % RH

Ketone	Sample	Mixture, 20 ppb, 80 % RH, 100 mL/min for 8 hours		
		5 ºC	25 ºC	40 ºC
Acetoin	1	64.4	83.3	91.4
	2	56.6	90.5	94.5
	3	68.4	81.4	109.3
	Mean	63.1	85.1	98.4
	SD	6.0	4.8	9.5
	CV	9.5	5.6	9.7
Diacetyl	1	86.67	93.1	99.8
	2	86.41	93.1	104.6
	3	87.79	97.1	103.4
	Mean	86.96	94.4	102.6
	SD	0.74	2.3	2.5
	CV	0.85	2.5	2.4
2,3-Pentanedione	1	83.7	89.3	86.5
	2	76.6	86.8	86.3
	3	75.2	82.6	92.2
	Mean	78.5	86.3	88.3
	SD	4.5	3.4	3.3
	CV	5.8	3.9	3.8

Table 7-21: Acetoin Sample Recoveries (%) of 80 ppb at 25 ºC and 5 % RH Sampled for 1 Hour at 100 mL/min at Different Storage Conditions

Period (day)	Temp (ºC)	Sample 1	Sample 2	Sample 3	Mean	SD	CV	p-Value
0	N/A	84.4	95.0	100.1	93.2	8.0	8.6	N/A
3	-20	85.7	90.0	83.1	86.3	3.5	4.0	0.2664
3	5	86.8	85.8	91.0	87.9	2.8	3.1	0.3920
3	25	91.2	97.9	100.8	96.6	4.9	5.1	0.5682
30	-20	109.4	100.9	93.7	101.4	7.8	7.7	0.2757
30	5	104.1	91.2	91.1	95.5	7.5	7.8	0.7350
30	25	98.3	101.5	89.7	96.5	6.1	6.3	0.5986

Table 7-22: Acetoin Sample Recoveries (%) of 80 ppb at 25 ºC and 80 % RH Sampled for 1 Hour at 100 mL/min at Different Storage Conditions

Period (day)	Temp (ºC)	Sample 1	Sample 2	Sample 3	Mean	SD	CV	p-Value
0	N/A	89.8	89.9	85.8	88.5	2.3	2.6	N/A
3	-20	82.6	91.3	86.4	86.8	4.4	5.1	0.5835
3	5	88.8	86.4	81.6	85.6	3.6	4.2	0.3230
3	25	80.9	81.3	87.0	83.0	3.4	4.1	0.0828
30	-20	78.3	81.4	81.7	80.5	1.9	2.4	0.0098*
30	5	76.4	78.3	79.1	77.9	1.4	1.7	0.0065*
30	25	90.4	88.0	91.8	90.1	1.9	2.1	0.4212

*: Significantly different at $p \leq 0.05$ compared to day 0

Table 7-23: Diacetyl Sample Recoveries (%) of 80 ppb at 25 ºC and 5 % RH Sampled for 1 Hour at 100 mL/min at Different Storage Conditions

Period (day)	Temp (ºC)	Sample 1	Sample 2	Sample 3	Mean	SD	CV	p-Value
0	N/A	88.4	86.2	88.6	87.7	1.3	1.5	N/A
3	-20	90.8	80.4	77.3	82.8	7.1	8.6	0.3628
3	5	88.4	80.2	77.6	82.1	5.7	6.9	0.2350
3	25	94.0	81.4	80.3	85.2	7.6	9.0	0.6350
30	-20	97.7	84.8	81.6	88.0	8.5	9.7	0.9556
30	5**	73	61	51	62	11	17	0.0517
30	25	86.6	90.6	81.4	86.2	4.6	5.3	0.6454

**: Recovery was low due to abnormally high blank. When the mean value of the blanks at -20 and 25 ºC for 30 day storage was used, the recovery was 124 ± 14 % at 5 ºC for 30 day storage.

Table 7-24: Diacetyl Sample Recoveries (%) of 80 ppb at 25 ºC and 80 % RH Sampled for 1 Hour at 100 mL/min at Different Storage Conditions

Period (day)	Temp (ºC)	Sample 1	Sample 2	Sample 3	Mean	SD	CV	p-Value
0	N/A	85.1	78.9	86.6	83.5	4.1	4.9	N/A
3	-20	85.6	79.3	86.3	83.7	3.9	4.6	0.9584
3	5	88.0	76.8	79.0	81.3	6.0	7.3	0.6150
3	25	88.3	80.4	78.9	82.6	5.0	6.1	0.8058
30	-20	100.9	87.3	89.6	92.6	7.3	7.9	0.1580
30	5**	65.7	64.1	55.2	61.7	5.6	9.2	0.0056*
30	25	98.9	92.4	82.1	91.1	8.5	9.3	0.2571

*: Significantly different at $p \leq 0.05$ compared to day 0

**: Recovery was low due to abnormally high blank. When the mean value of the blanks at -20 and 25 ºC for 30 day storage was used, the recovery was 139 ± 20 % at 5 ºC for 30 day storage.

Table 7-25: 2,3-Pentanedione Sample Recoveries (%) of 80 ppb at 25 ºC and 5 % RH Sampled for 1 Hour at 100 mL/min at Different Storage Conditions

Period (day)	Temp (ºC)	Sample 1	Sample 2	Sample 3	Mean	SD	CV	p-Value
0	N/A	91.3	92.9	95.0	93.1	1.8	1.9	N/A
3	-20	83.2	89.1	75.7	82.7	6.7	8.1	0.1229
3	5	80.1	85.9	83.6	83.2	2.9	3.5	0.0155*
3	25	86.3	99.5	85.0	90.2	8.0	8.9	0.6098
30	-20	91.0	83.3	79.7	84.7	5.8	6.8	0.1380
30	5	85.5	79.4	84.0	83.0	3.2	3.9	0.0177*
30	25	77.2	80.7	78.5	78.8	1.7	2.2	0.0006*

*: Significantly different at $p \leq 0.05$ compared to day 0

Table 7-26: 2,3-Pentanedione Sample Recoveries (%) of 80 ppb at 25 ºC and 80 % RH Sampled for 1 Hour at 100 mL/min at Different Storage Conditions

Period (day)	Temp (ºC)	Sample 1	Sample 2	Sample 3	Mean	SD	CV	p-Value
0	N/A	90.9	83.8	88.3	87.7	3.6	4.1	N/A
3	-20	83.0	84.4	82.5	83.3	1.0	1.2	0.1807
3	5	87.8	84.1	82.2	84.7	2.8	3.3	0.3308
3	25	84.5	87.4	78.7	83.5	4.4	5.3	0.2789
30	-20	79.2	79.7	84.2	81.1	2.7	3.4	0.0652
30	5	77.7	80.0	81.6	79.8	1.9	2.4	0.0445*
30	25	75.5	79.8	83.9	79.7	4.2	5.2	0.0678

*: Significantly different at $p \leq 0.05$ compared to day 0

Table 7-27: D-Diacetyl Sample Recoveries (%) of 80 ppb at 25 ºC and 5 % RH Sampled for 1 Hour at 100 mL/min at Different Storage Conditions

Period (day)	Temp (ºC)	Sample 1	Sample 2	Sample 3	Mean	SD	CV
0	N/A	42.7	42.5	38.8	41.3	2.2	5.4
3	-20	42.2	44.4	36.6	41.1	4.0	9.8
3	5	49.6	48.6	45.9	48.0	1.9	4.0
3	25	61.7	68.2	57.5	62.5	5.4	8.6
30	-20	65	56	44	55	10	19
30	5	73	61	51	62	11	17
30	25	70.8	70.7	59.1	66.8	6.7	10

Table 7-28: D-Diacetyl Sample Recoveries (%) of 80 ppb at 25 ºC and 80 % RH Sampled for 1 Hour at 100 mL/min at Different Storage Conditions

Period (day)	Temp (ºC)	Sample 1	Sample 2	Sample 3	Mean	SD	CV
0	N/A	38.6	36.4	31.0	35.3	3.9	11
3	-20	42.8	41.5	39.8	41.4	1.5	3.6
3	5	53.4	46.0	40.0	46.5	6.7	14
3	25	52.1	45.2	43.0	46.8	4.8	10
30	-20	61.5	53.2	52.6	55.8	5.0	9.0
30	5	65.7	64.1	55.2	61.7	5.6	9.2
30	25	78.1	69.4	63.6	70.4	7.3	10

Table 7-29: D-2,3-Pentanedione Sample Recoveries (%) of 80 ppb at 25 ºC and 5 % RH Sampled for 1 Hour at 100 mL/min at Different Storage Conditions

Period (day)	Temp (ºC)	Sample 1	Sample 2	Sample 3	Mean	SD	CV
0	N/A	27.0	26.0	24.8	25.9	1.1	4.2
3	-20	22.5	27.6	24.7	24.9	2.5	10
3	5	33.0	35.5	39.0	35.8	3.0	8.4
3	25	47.6	54.6	51.0	51.1	3.5	6.8
30	-20	45.7	45.0	37.2	42.7	4.7	11
30	5	57.0	55.1	51.2	54.4	3.0	5.5
30	25	63.6	66.1	62.4	64.0	1.9	3.0

Table 7-30: D-2,3-Pentanedione Sample Recoveries (%) of 80 ppb at 25 ºC and 80 % RH Sampled for 1 Hour at 100 mL/min at Different Storage Conditions

Period (day)	Temp (ºC)	Sample 1	Sample 2	Sample 3	Mean	SD	CV
0	N/A	19.8	22.3	20.8	20.9	1.2	5.8
3	-20	22.3	23.7	27.9	24.7	2.9	12
3	5	31.0	34.0	32.3	32.5	1.5	4.7
3	25	34.20	34.68	35.88	34.92	0.86	2.5
30	-20	40.6	43.3	45.2	43.0	2.3	5.3
30	5	49.9	56.8	53.6	53.5	3.5	6.5
30	25	63.8	67.3	66.2	65.8	1.8	2.7

Table 7-31: Calibration of Anemometer at 25 ºC and 760 mm Hg

Q (mL/min)				CV	V (ft/min)				CV	Error
Before	After	Mean	SD	(%)	Exp	Obs	Ave	SD	(%)	(%)
0.0	0.00	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0
0.0	0.00				0.0	0				
0.0	0.00				0.0	0				
104.0	95.96	100.0	3.4	3.4	10	10	10.7	1.2	11	2.9
103.0	97.43				10	12				
102.0	97.74				10	10				
304.5	305.0	304.9	1.2	0.39	32	35	35.7	1.2	3.2	13
303.5	306.6				32	37				
303.8	305.8				32	35				
502.2	497.2	499.0	1.9	0.39	52	51	50.3	1.2	2.3	-2.6
499.5	499.8				52	49				
497.7	497.3				52	51				
1005	1001	1001.3	2.1	0.21	104	110	110.0	2.0	1.8	6.0
1001	1000				104	108				
1002	998.7				104	112				

Table 7-32: Calibration of Syringe Pump using a 5-mL Gas Tight Syringe at 25 ºC and 760 mm Hg

Flow Rate (mL/hr)	Water Loss in 1 hr		
	Mass (g)	Volume (mL)	Error (%)
0.10	0.0999	0.1002	0.23
0.30	0.3001	0.3010	0.32
0.50	0.4977	0.4992	-0.15
0.70	0.6984	0.7005	0.072
1.00	1.002	1.005	0.48

Table 7-33: Calibration of Syringe Pump using a 50-mL Gas Tight Syringe 25 ºC and 760 mm Hg

Flow Rate (mL/hr)	Water Loss in 1 hr		
	Mass (g)	Volume (mL)	Error (%)
2.0	1.955	1.961	-2.0
3.5	3.470	3.480	-0.56
5.0	4.904	4.919	-1.6
6.5	6.274	6.293	-3.2
8.0	7.683	7.706	-3.7

Table 7-34: PFBHA O-Oxime Liquid/Solid Spiking Recoveries (%) with the Optimized Tenax-Water-Hexane Method over the 1-20 ppb Ketone Equivalent Range

PFBHA O-Oxime	Sample	Equivalent Concentration (ppb)			
		1	5	10	20
M-Acetoin	1	85.3	89.8	84.8	87.6
	2	91.0	85.1	88.0	83.1
	3	80.2	90.1	83.3	94.8
	Mean	85.5	88.3	85.4	88.5
	SD	5.4	2.8	2.4	5.9
	CV	6.3	3.1	2.8	6.6
D-Diacetyl	1	82.0	83.8	80.8	86.8
	2	83.4	89.4	89.1	87.5
	3	89.0	83.0	86.8	84.9
	Mean	84.8	85.4	85.6	86.4
	SD	3.7	3.5	4.3	1.4
	CV	4.3	4.1	5.0	1.6
D-2,3-Pentanedione	1	97.3	92.5	86.4	94.19
	2	103.3	97.9	97.4	94.51
	3	103.4	88.7	94.7	92.90
	Mean	101.3	93.0	92.8	93.87
	SD	3.5	4.7	5.7	0.85
	CV	3.4	5.0	6.2	0.91

Table 7-35: Ketone Liquid/Solid Spiking Recoveries (%) with Tenax-Water-Hexane Method over the 1-40 ppb Ketone Equivalent Range

Ketone	Sample	Equivalent Concentration (ppb)					
		1	5	10	20	30	40
Acetoin	1	70.9	81.8	83.0	80.6	75.1	79.3
	2	79.9	93.8	80.3	81.1	80.4	85.6
	3	77.1	81.4	81.4	83.6	84.6	84.2
	Mean	75.9	85.7	81.5	81.8	80.0	83.0
	SD	4.6	7.0	1.4	1.6	4.7	3.3
	CV	6.1	8.2	1.7	2.0	5.9	4.0
Diacetyl	1	223	77.4	80.79	94.0	87.3	91.6
	2	162	80.8	80.93	96.7	96.8	100.4
	3	183	84.7	81.85	100.9	103.2	98.9
	Mean	189*	81.0	81.19	97.2	95.8	97.0
	SD	31	3.7	0.58	3.4	8.0	4.8
	CV	17**	4.5	0.71	3.5	8.4	4.9
2,3-Pentanedione	1	172.7	77.4	80.67	78.9	73.8	80.0
	2	167.6	87.9	81.40	79.3	83.3	88.2
	3	170.7	82.5	81.34	85.6	89.4	88.3
	Mean	170.4*	82.6	81.13	81.3	82.2	85.5
	SD	2.6	5.2	0.41	3.7	7.8	4.8
	CV	1.5	6.3	0.50	4.6	9.5	5.6

*: different from 75 to 125 % recovery
**: CV > 10 %

Table 7-36: Acetoin Sample Recoveries (%) of 80 ppb at 25 ºC and 5 % RH Sampled for 1 Hour at Different Storage Conditions

Period (day)	Temp (ºC)	Sample 1	Sample 2	Sample 3	Mean	SD	CV	p-Value
0	N/A	111.5	123.7	113.2	116.1	6.6	5.7	N/A
3	-20	102.0	114.5	110.5	109.0	6.4	5.9	0.2535
3	25	102.1	105.0	117.4	108.2	8.1	7.5	0.2600
30	-20	121.5	118.7	120.4	120.2	1.4	1.2	0.4047
30	25	120.3	124.0	119.9	121.4	2.3	1.9	0.3196

Table 7-37: Diacetyl Sample Recoveries (%) of 80 ppb at 25 ºC and 5 % RH Sampled for 1 Hour at Different Storage Conditions

Period (day)	Temp (ºC)	Sample 1	Sample 2	Sample 3	Mean	SD	CV	p-Value
0	N/A	82.8	100.5	90.6	91.3	8.9	9.7	N/A
3	-20	84.9	102.8	94.3	94.0	9.0	9.5	0.7345
3	25	95.3	90.9	103.6	96.6	6.4	6.7	0.4533
30	-20	87.3	90.2	101.6	93.1	7.6	8.1	0.8093
30	25	83.4	83.3	79.9	82.2	2.0	2.5	0.2254

Table 7-38: 2,3-Pentanedione Sample Recoveries (%) of 80 ppb at 25 ºC and 5 % RH Sampled for 1 Hour at Different Storage Conditions

Period (day)	Temp (ºC)	Sample 1	Sample 2	Sample 3	Mean	SD	CV	p-Value
0	N/A	114.1	103.5	108.6	108.7	5.3	4.9	N/A
3	-20	92.9	95.8	83.5	90.7	6.4	7.1	0.0202*
3	25	77.0	76.0	86.2	79.7	5.6	7.1	0.0029*
30	-20	98.3	96.5	94.5	96.4	1.9	1.9	0.0639
30	25	87.3	83.5	75.1	82.0	6.3	7.6	0.0049*

*: Significantly different at $p \leq 0.05$ compared to day 0

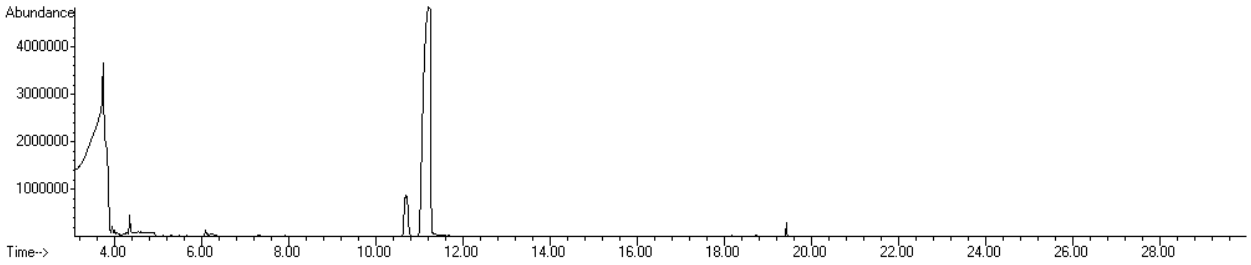


Figure 7-1: Gas Chromatogram Using TIC for M-Diacetyl in Hexane for 3 Days

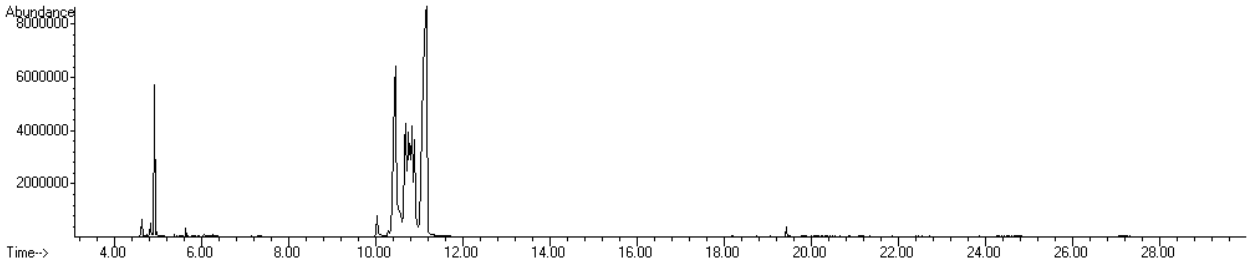


Figure 7-2: Gas Chromatogram Using TIC for M-Diacetyl in Ethanol for 3 Days

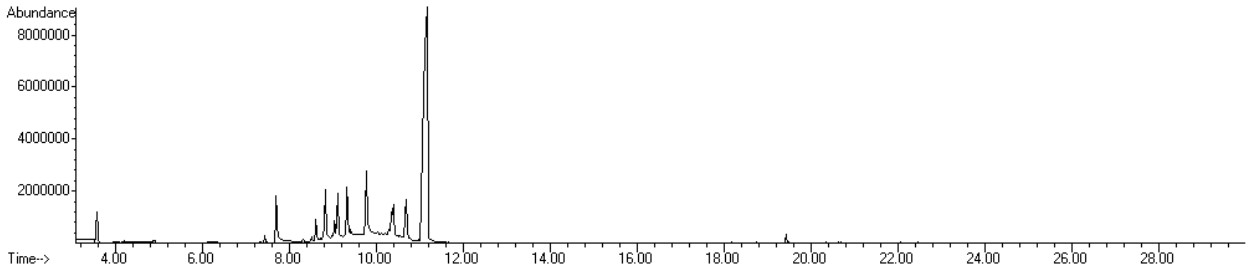


Figure 7-3: Gas Chromatogram Using TIC for M-Diacetyl in Acetonitrile for 3 Days

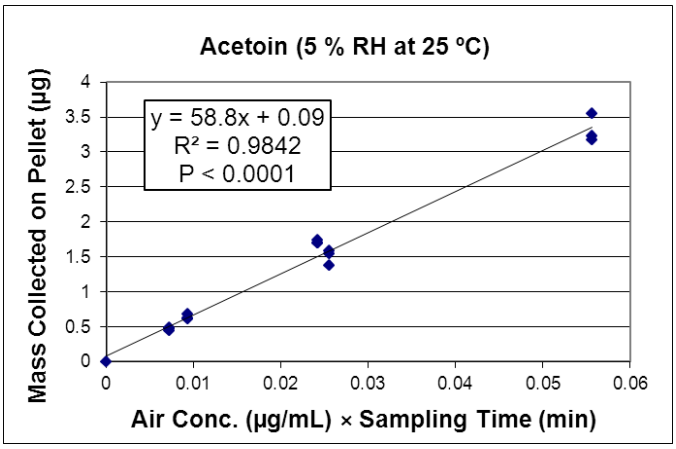


Figure 7-4: Acetoin Experimental Sampling Constant at 5 % RH and 25 ºC

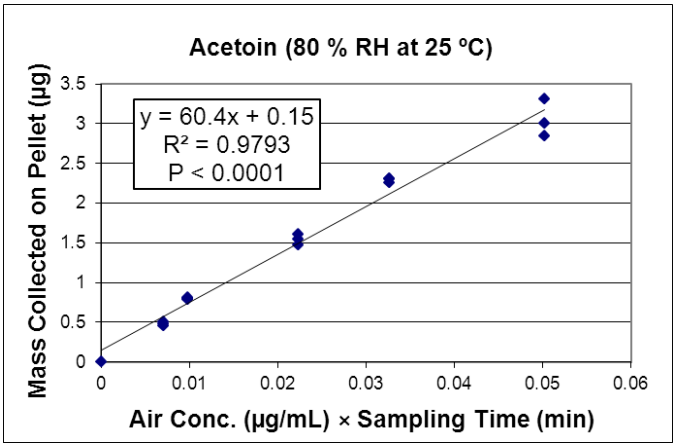


Figure 7-5: Acetoin Experimental Sampling Constant at 80 % RH and 25 ºC

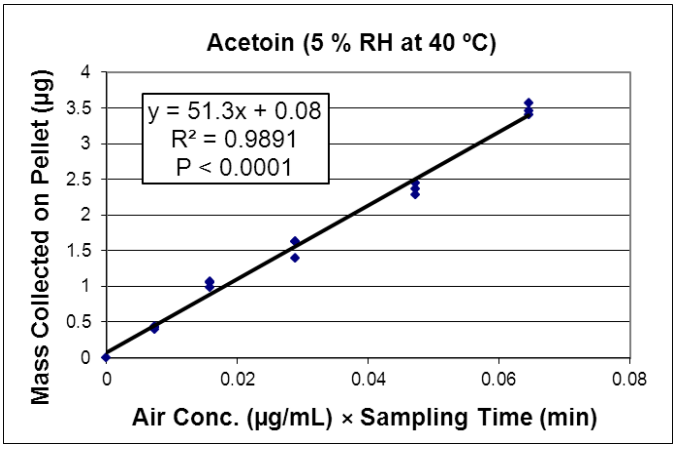


Figure 7-6: Acetoin Experimental Sampling Constant at 5 % RH and 40 ºC

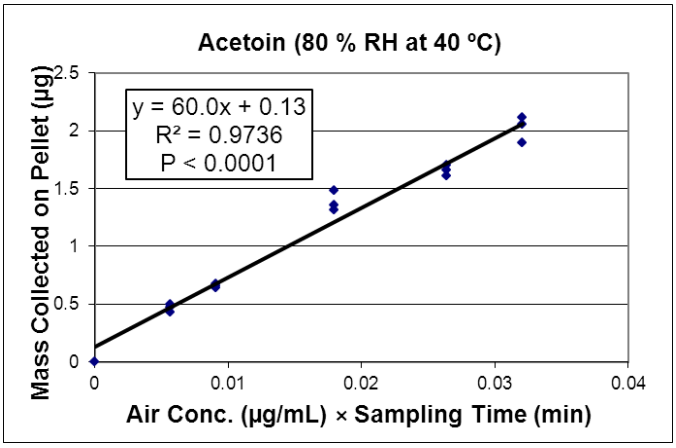


Figure 7-7: Acetoin Experimental Sampling Constant at 80 % RH and 40 ºC

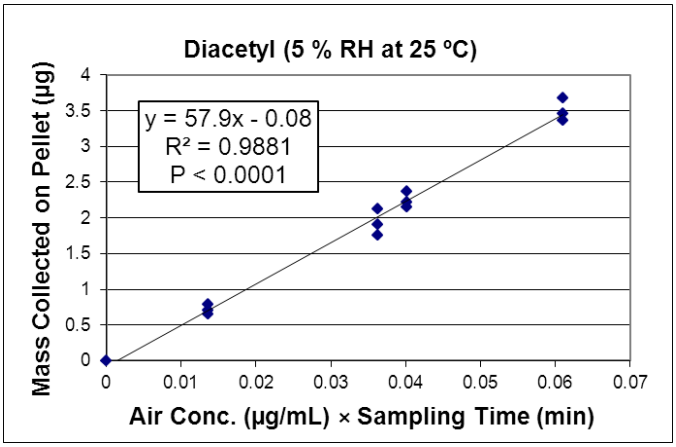


Figure 7-8: Diacetyl Experimental Sampling Constant at 5 % RH and 25 ºC

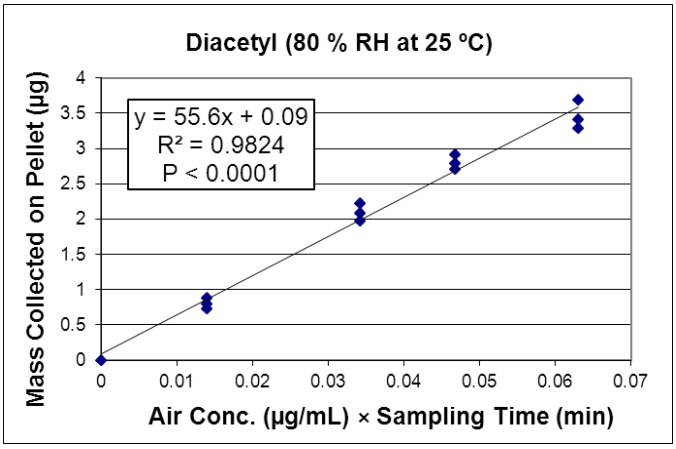


Figure 7-9: Diacetyl Experimental Sampling Constant at 80 % RH and 25 ºC

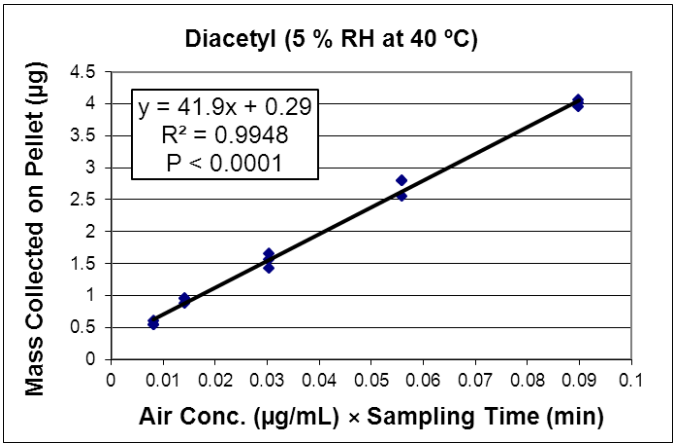


Figure 7-10: Diacetyl Experimental Sampling Constant at 5 % RH and 40 ºC

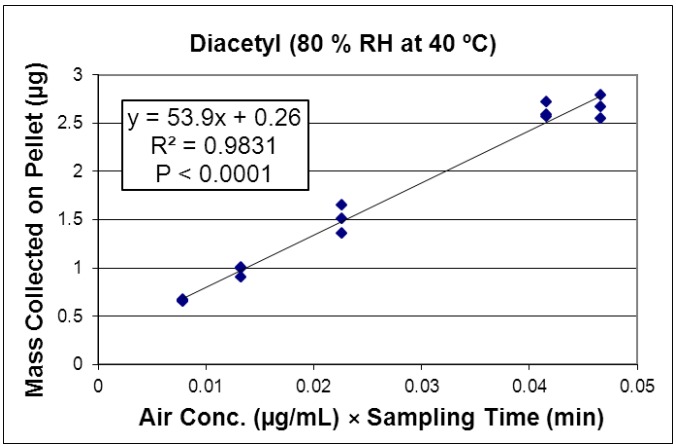


Figure 7-11: Diacetyl Experimental Sampling Constant at 80 % RH and 40 ºC

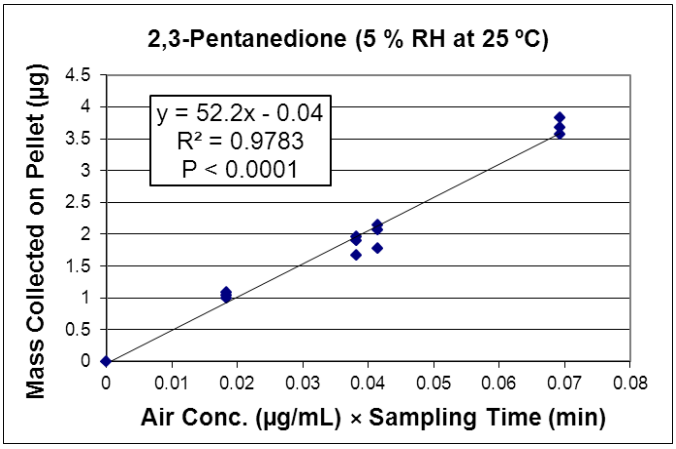


Figure 7-12: 2,3-Pentanedione Experimental Sampling Constant at 5 % RH and 25 ºC

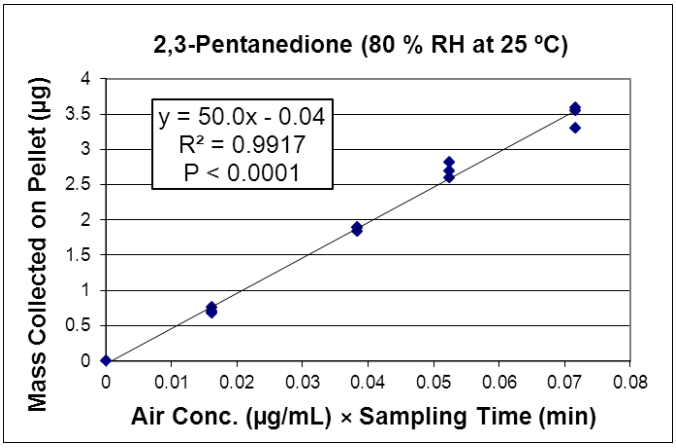


Figure 7-13: 2,3-Pentanedione Experimental Sampling Constant at 80 % RH and 25 ºC

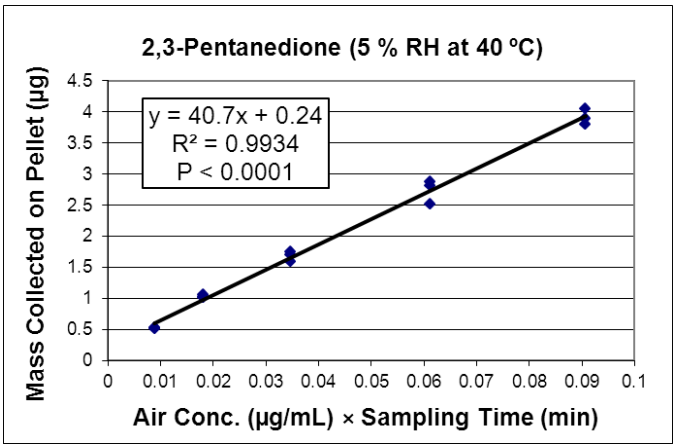


Figure 7-14: 2,3-Pentanedione Experimental Sampling Constant at 5 % RH and 40 ºC

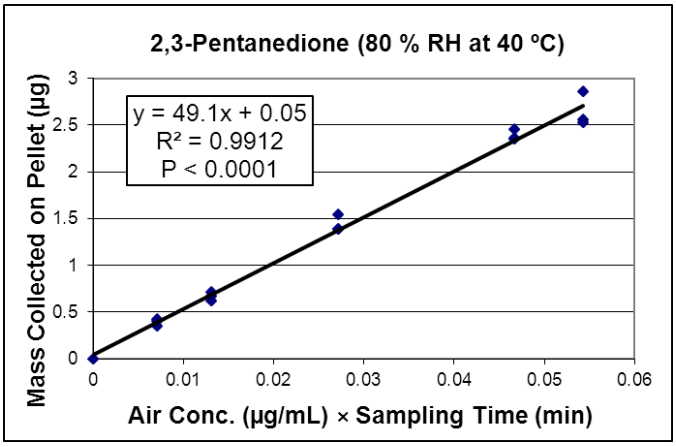


Figure 7-15: 2,3-Pentanedione Experimental Sampling Constant at 80 % RH and 40 ºC

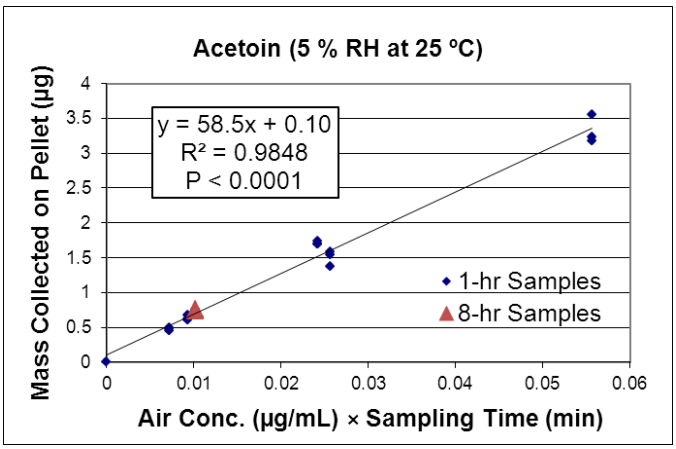


Figure 7-16: Acetoin Experimental Sampling Constant at 5 % RH and 25 ºC with 1 and 8-Hour Samples

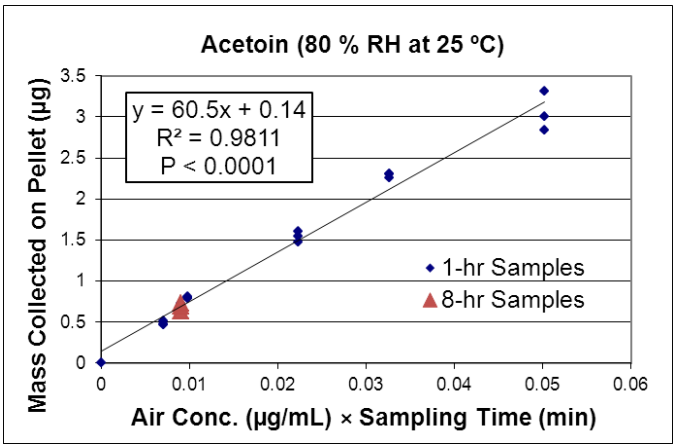


Figure 7-17: Acetoin Experimental Sampling Constant at 80 % RH and 25 ºC with 1 and 8-Hour Samples

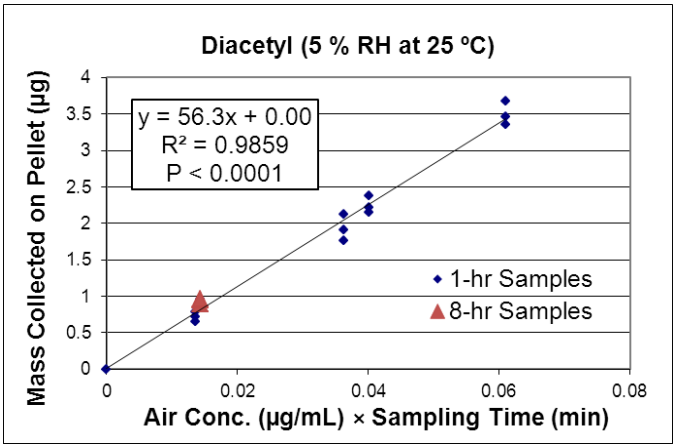


Figure 7-18: Diacetyl Experimental Sampling Constant at 5 % RH and 25 ºC with 1 and 8-Hour Samples

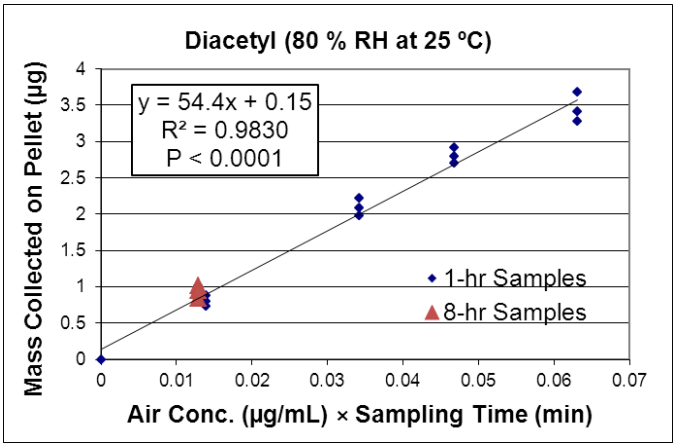


Figure 7-19: Diacetyl Experimental Sampling Constant at 80 % RH and 25 ºC with 1 and 8-Hour Samples

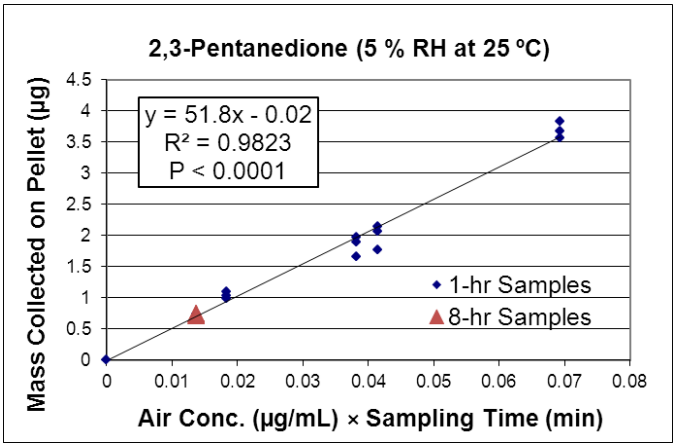


Figure 7-20: 2,3-Pentanedione Experimental Sampling Constant at 5 % RH and 25 ºC with 1 and 8-Hour Samples

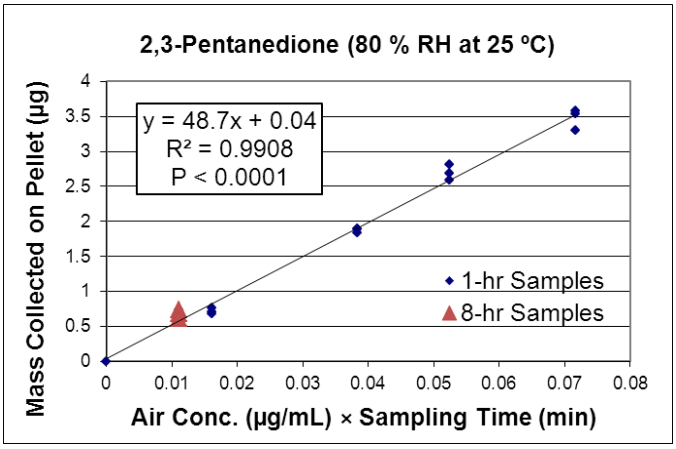


Figure 7-21: 2,3-Pentanedione Experimental Sampling Constant at 80 % RH and 25 ºC with 1 and 8-Hour Samples

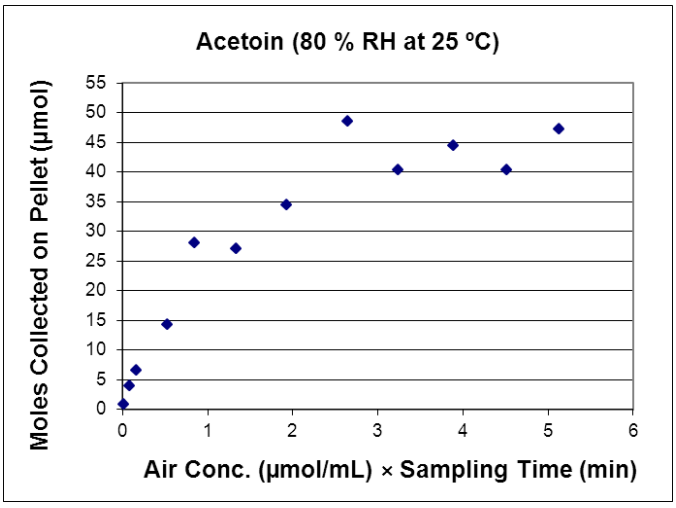


Figure 7-22: Pellet Molar Capacity for Acetoin at 80 % RH at 25 ºC

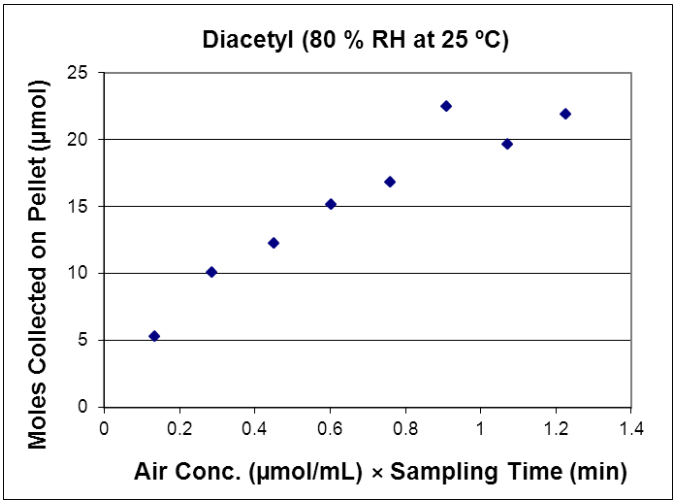


Figure 7-23: Pellet Molar Capacity for Diacetyl at 80 % RH at 25 ºC

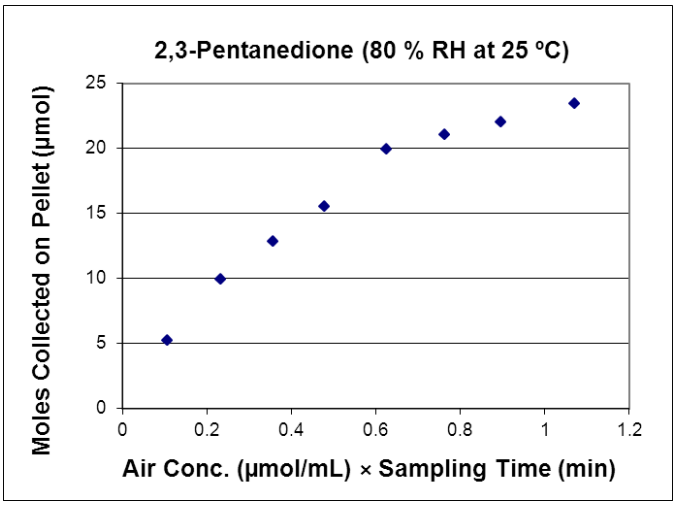


Figure 7-24: Pellet Molar Capacity for 2,3-Pentanedione at 80 % RH at 25 ºC

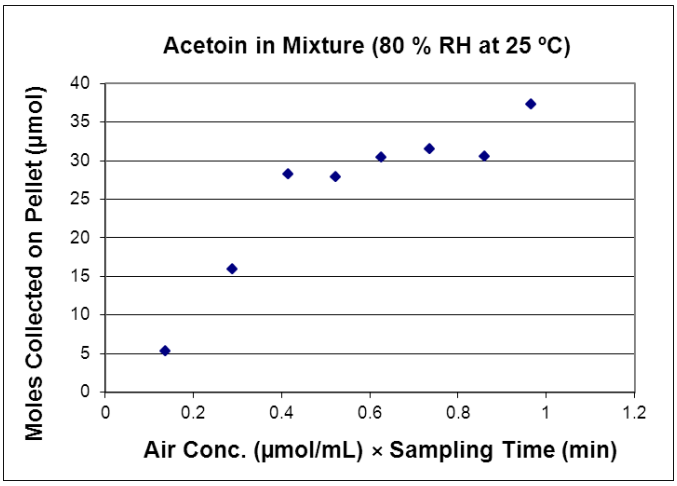


Figure 7-25: Pellet Molar Capacity for Acetoin at 80 % RH as a Vapor Mixture at 25 ºC

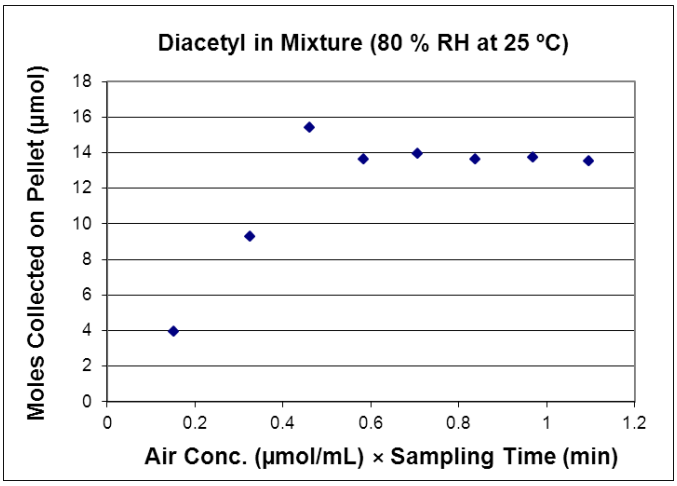


Figure 7-26: Pellet Molar Capacity for Diacetyl at 80 % RH as a Vapor Mixture at 25 ºC

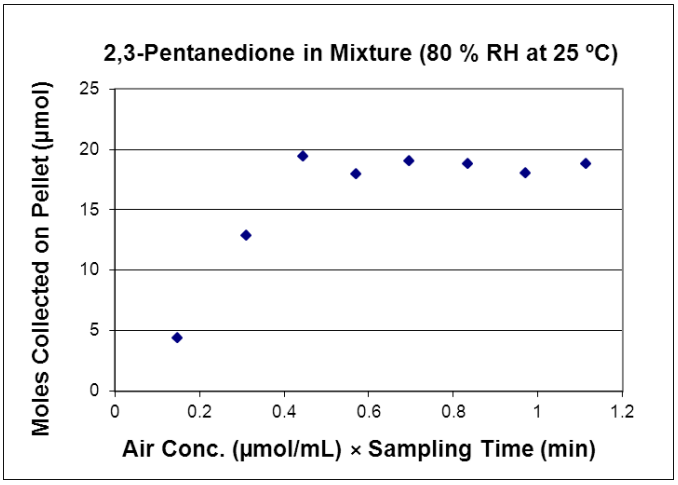


Figure 7-27: Pellet Molar Capacity for 2,3-Pentanedione at 80 % RH as a Vapor Mixture at 25 ºC

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