Chemical Information Review Document

for

Artificial Butter Flavoring

and Constituents
Diacetyl [CAS No. 431-03-8] and Acetoin [CAS No. 513-86-0]

Supporting Nomination for Toxicological Evaluation by the National Toxicology Program

January 2007

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Under Contract No. N01-ES-35515

Prepared for:
National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health
U.S Department of Health and Human Services
Research Triangle Park, NC
http://ntp.niehs.nih.gov/

Abstract

Artificial butter flavoring and two important constituents, diacetyl and acetoin, were nominated by the United Food and Commercial Workers International Union for long-term testing via inhalation for respiratory and other toxicity and for cancer-causing properties. There is growing concern that workers in the microwave popcorn manufacturing industry may be at risk of developing the lung disease bronchiolitis obliterans from exposure to vapors from artificial butter flavoring. The first case of bronchiolitis obliterans in a popcorn manufacturing worker was reported in 2000 in Missouri. Since then several devastating outbreaks of severe and even fatal lung disease, including bronchiolitis obliterans, have been documented among workers in microwave popcorn manufacturing plants who have been exposed to the vapors of butter flavoring. To date, limited toxicological studies are available for artificial butter flavoring and its constituents. Inhalation studies in male rats showed that exposure to vapors from artificial butter flavoring caused necrosis of nasal and airway epithelium. Necrotizing bronchitis was observed in the lung, and necrosuppurative rhinitis and inflammation were seen at all nasal levels. An inhalation study with diacetyl also produced significant necrosis of nasal epithelium and significant necrosis of tracheal epithelium in male rats. However, no significant effects in the lung were reported. In another inhalation study, necropsy revealed general congestion, focal hyperemia of the lungs, atelectasis and bloody edema of the lungs, bronchial edema, and intensified hydrothorax in rats that did not survive a four hour treatment with diacetyl. Histopathological examination showed emphysema, hyperemia of the lungs, peripheral or centrilobular swelling of hepatocytes, and necrosis in the proximal tubules of the kidney. In rats, [^{14}C]-diacetyl given by intragastric gavage was rapidly metabolized and excreted, primarily as carbon dioxide in the breath and in urine. Metabolism of acetoin *in vivo* was mainly by oxidation at low concentrations and by reduction to 2,3-butanediol at higher concentrations. Diacetyl was not a reproductive or teratological toxicant in studies with pregnant mice, rats, or hamsters. Intraperitoneal injections of diacetyl or acetoin did not induce tumors in mice. Diacetyl, but not acetoin, was mutagenic in some strains of bacteria. It was negative in a micronucleus test but induced sister chromatid exchanges in Chinese hamster ovary AUXB1 cells and unscheduled DNA synthesis in various organs of laboratory animals. Diacetyl exhibited activating and deactivating effects on several enzymes and metabolic processes. It has been postulated that oxidative stress may play a role in diacetyl-induced lung damage.

Executive Summary

Basis for Nomination

Artificial butter flavoring and two important constituents, diacetyl and acetoin, were nominated by the United Food and Commercial Workers International Union (UFCW) for long-term testing via inhalation for respiratory and other toxicity and for cancer-causing properties. After the first incidence of bronchiolitis obliterans associated with microwave popcorn manufacturing in Jasper, MO was reported in 2000, several devastating outbreaks of severe and even fatal lung disease, including bronchiolitis obliterans, have been documented among workers in other microwave popcorn plants who had been exposed to the vapors of butter flavoring. These outbreaks have been reported in the scientific literature and the popular press. Since butter flavoring mixtures consist of more than 100 different chemicals, the most prominent being diacetyl and acetoin, the UFCW also recommends that the flavoring mixture as a whole be tested and that the National Toxicology Program (NTP) explore the effects of compounds with chemical and physical properties similar to diacetyl and acetoin.

Nontoxicological Data

Diacetyl is naturally found in foods and is also used as a synthetic flavoring agent and an aroma carrier in foods, including butter, caramel, vinegar, dairy products, and coffee. Acetoin is used as a fragrance carrier and in the preparation of flavors and essences; it is found in many of the same foodstuffs as diacetyl (e.g., butter, corn, wine, and cocoa). In the United States, diacetyl and acetoin are regulated by the Food and Drug Administration as substances directly added to human food and are generally recognized as safe. Both compounds can be detected using gas chromatography, usually coupled with flame ionization detection. Diacetyl is available form several U.S. suppliers and is produced in different ways, including converting methyl ethyl ketone to an isonitroso compound, followed by hydrolyzation with hydrochloric acid or by oxidation of 2-butanone over a copper catalyst. Diacetyl is also a product of fermentation of glucose via methylacetylcarbinol and of lactic acid bacteria activity during the production of beer. Acetoin is prepared from diacetyl by partial reduction with zinc and acid. It is also produced by the action of sorbose bacteria or *Mycoderma aceti* on 2,3-butanediol or by the action of fungi on sugar cane juice. It is a by-product of fermentation and preparation of cream for churning. Diacetyl has been identified in aroma components of tobacco smoke and in several plants. It photodegrades quickly in the atmosphere and is not likely to absorb significantly in soil or sediment or to bioconcentrate in fish. Acetoin gradually oxidizes to diacetyl in air and forms a solid dimer on standing or treatment with granulated zinc. It has high mobility if released to soil and is not expected to absorb to suspended solids or sediments or to undergo direct photolysis. Like diacetyl, it has a low potential for bioconcentration in aquatic organisms.

Human Data

Diacetyl and acetoin are formed endogenously in humans from decarboxylation of pyruvate. They are important volatile organic compounds (VOCs) emitted from butter flavoring and are of concern to workers in the microwave popcorn production industry. Case reports of severe bronchiolitis obliterans syndrome in eight former workers at a Missouri microwave popcorn plant (Gilster-Mary Lee Corporation) sparked public interest in May 2000. NIOSH reported that workers from three other microwave popcorn plants had been exposed to diacetyl and other VOCs form butter flavoring mixtures and had developed occupational lung disease; at least three deaths were reported among these individuals. Bronchiolitis obliterans has therefore been called "popcorn worker's lung" or "popcorn packers' workers' lung." Of the eight workers at the Missouri plant, four worked in the plant's production area (included a mixing room) and four worked in the packaging areas. Workers in these sections were exposed to 800x and 15x the mean atmospheric concentration of diacetyl, respectively, compared to office, warehouse, and outside areas. Workers in the production areas also had significantly higher rates of shortness of breath on exertion, breathing problems, a combination of respiratory symptoms, unusual fatigue, other systemic symptoms, and rashes or other skin problems. As cumulative exposure to diacetyl increased, the

incidence of airway obstruction increased. The data suggested diacetyl as a cause of respiratory disease or a marker of the causative exposure. However, workers in the production areas were also exposed to the highest concentrations of ketones, other VOCs, and respirable dust. Bronchiolitis obliterans has also been reported in workers of other industries (baking industry, flavoring manufacturing plants, and snack food manufacturer using flavorings or spices).

Workers exposed to butter flavoring vapors have also reported eye (chemical burns), skin, and nasal irritations. Patch testing and maximization testing with diacetyl produced no irritation or sensitization, respectively, in volunteers. Tests with acetoin also resulted in no irritation or sensitization reactions.

Toxicological Data

No short-term/subchronic or chronic inhalation studies were available for artificial butter flavorings, diacetyl, or acetoin. Additionally, data regarding initiation/promotion, anticarcinogenicity, and immunotoxicity were not found.

Chemical Disposition, Metabolism, and Toxicokinetics

Metabolic interconversion between diacetyl, acetoin and 2,3-butanediol has been observed using rat liver extracts.

When administered to male Fischer 344 rats via intragastric gavage, a single dose of radiolabeled [^{14}C]-diacetyl (1.58, 15.8, or 158 mg/kg [0.0184, 0.184, or 1.84 mmol/kg]) resulted in excretion of 82.0, 72.7, and 54.3% of the administered doses, respectively, as carbon dioxide at 72 hours. In urine, the excreted amounts were 6.86, 15.7, and 34.1%, respectively. At all tested levels, total excretion of radioactivity in urine, feces, and expired breath accounted for 86-87% of the dose recovered within 24 hours. In normal rat liver mitochondria, diacetyl uncoupled oxidative phosphorylation, totally eliminated respiratory control with substrates, and partially eliminated it with succinate.

Metabolism of acetoin *in vivo* is mainly by oxidation at low concentrations and by reduction to 2,3-butanediol at high concentrations. When acetoin (doses not provided) was i.p. injected into albino rats, ^{12}C-carbon dioxide was found in expired air (average of 15% during 12 hours). When acetoin was administered orally or subcutaneously to rats, no diacetyl and very little acetoin were detected in the urine; 2,3-butanediol was the major excretion product. In rabbits orally given acetoin and in rabbit liver homogenate incubated with acetoin, acetylation was increased. In male guinea pigs, acetoin was an intermediary metabolite in the reduction of methyl ethyl ketone to 2,3-butanediol. In rat and rabbit liver extracts, >95% of radiolabeled [2,3-^{14}C]-acetoin was detected as a mixture of 2,3-butanediol stereoisomers.

Acute Toxicity

The LC_{50} value for diacetyl in rats was reported between 2.25 and 5.2 mg/L (639 and 1477 ppm) for a four-hour period. Diacetyl was a severe skin and eye irritant in rabbits. Acetoin was a moderate irritant on intact and abraded skin of rabbits; an LD_{50} value >5000 mg/kg (56.75 mmol/kg) was calculated.

Male rats exposed to vapors from artificial butter flavoring (average diacetyl concentrations: 203, 285, 352 [constant], or 371 [pulsed] ppm; range 72-940 ppm) for six hours exhibited necrosis of nasal and airway epithelium. At levels of 285-371 ppm diacetyl, necrotizing bronchitis was observed in the lung; at 203-371 ppm diacetyl, necrosuppurative rhinitis and inflammation were seen at all nasal levels.

Inhalation of diacetyl (99, 198, or 295 ppm [349, 697, or 1039 mg/m^3]) for six hours also produced significant necrosis of nasal epithelium at ≥198 ppm and significant necrosis of tracheal epithelium at the high dose in male rats. No significant effects in the lung were reported. When tested in male and female Wistar rats, inhalation of diacetyl FCC (2.25, 5.2, and 23.9 mg/L [639.0, 1477, or 6788 ppm]) for four

hours resulted in deaths at the mid and high doses. Necropsy showed general congestion in dead rats, focal hyperemia of the lungs and empty gastrointestinal tract in mid-dose animals, and atelectasis and bloody edema of the lungs, bronchial edema, and intensified hydrothorax in high-dose rats. Histopathological examination revealed moderate emphysema and focal hyperemia of the lungs as well as peripheral swelling of hepatocytes at the mid dose, and widespread hyperemia of the lung, necrosis in the proximal tubules of the kidney, and centrilobular swelling of hepatocytes at the high dose.

No acute inhalation studies for acetoin were available.

Synergistic/Antagonistic Effects

When acetoin and ethanol were i.p. administered simultaneously in rats to cause loss of righting reflex and respiratory failure, the concentrations of both chemicals were additive in blood.

Cytotoxicity

Diacetyl (0.001, 0.1, or 1 mM [0.086, 8.6, or 86 µg/mL]) inhibited cell growth in ascites sarcoma cells by 37% at the mid dose and by 100% at the high dose.

Reproductive and Teratological Effects

When given via oral intubation to pregnant mice for ten days, diacetyl (1.6 g starter distillate/kg) had no effects on maternal or fetal survival or nidation and caused no statistically significant changes in the number of fetal abnormalities compared to controls. Tests in hamsters and rats gave similar results.

Carcinogenicity

When given i.p. to mice once weekly for 24 weeks, diacetyl (1.70 or 8.40 mg/kg [0.0197 or 0.0976 mmol/kg]) did not induce any lung tumors. Acetoin (total doses of 12.0 or 60.0 g/kg [136 or 681 mmol/kg] given i.p. 3x/week for 6-7 weeks) also showed no carcinogenic activity.

Genotoxicity

In several bacterial assays, diacetyl generally showed mutagenic activity in *Salmonella typhimurium* strains TA100, 102, and 104 with and without metabolic activation but none against strain TA98. Conflicting results were obtained in *Escherichia coli* strain WP2 uvra, but nonmutagenicity was demonstrated in the SOS-chromotest using *E. coli* PQ37. Diacetyl was also negative in a micronucleus test using mouse bone marrow cells. It induced sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) AUXB1 cells and unscheduled DNA synthesis in various organs of laboratory animals.

Acetoin (up to 4500 mg/plate [51.08 mmol/plate]) was generally nonmutagenic in bacteria *in vitro*.

Cogenotoxicity

Diacetyl induced mitotic chromosome loss in *Saccharomyces cerevisiae* in the presence of propionitrile.

Antigenotoxicity

In CHO AUXB1 cells, bisulfite significantly reduced the frequency of SCEs and proportion of endoreduplicated cells when diacetyl was administered. Sodium sulfite and heterocyclic amines inactivated the mutagenicity of diacetyl in *S. typhimurium* strain TA100.

Other Data

Effects on Enzymes

When administered to rats via gastric intubation, diacetyl (300 or 1500 mg/kg bw [3.48 or 17.42 mmol/kg bw]) produced increases in ornithine decarboxylase activity and DNA synthesis in the pyloric mucosa. Diacetyl has activating and deactivating effects on several enzymes and metabolic processes, including inactivating estradiol 17β-dehydrogenase in human placenta under ultraviolet light. In the lysosomal

enzyme α-*L*-iduronidase, it reduced the internalization of the enzyme into human diploid fibroblasts by 50% without affecting enzyme activity and reduced binding to the fibroblast membranes by 90%.

Possible Mechanism for Lung Damage by Diacetyl

Diacetyl may induce lung damage by oxidative stress. Lung injury was induced by phosgene and mustard via several processes, including free radical generation. Lung damage caused by ozone has also been suggested from the formation of reactive free radicals. Reduction potentials for diacetyl and its iminium derivatives were found to be in the range favorable for catalytic electron transfer *in vivo*, which can cause oxidative stress via reaction oxygen species as a result of redox cycling.

Structure-Activity Relationships

Genetic and carcinogenic effects for several diacetyl analogs, such as methylglyoxal and acetaldehyde, are included in an earlier NTP chemical background document for diacetyl (http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/431-03-8.pdf).

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Tables:

1.0 Basis for Nomination

Artificial butter flavoring and two important constituents, diacetyl and acetoin, were nominated by the United Food and Commercial Workers International Union (UFCW) for long-term testing via inhalation for respiratory and other toxicity and for cancer-causing properties (http://defendingscience.org/case_studies/upload/Union_petitionto_NTP.pdf). After the first incidence of bronchiolitis obliterans associated with microwave popcorn manufacturing in Jasper, MO was reported in 2000, several devastating outbreaks of severe and even fatal lung disease, including bronchiolitis obliterans, have been documented among workers in other microwave popcorn plants who had been exposed to the vapors of butter flavoring. These outbreaks have been reported in the scientific literature and the popular press. Since butter flavoring mixtures consist of more than 100 different chemicals, the most prominent being diacetyl and acetoin, the UFCW also recommends that the flavoring mixture as a whole be tested and that the National Toxicology Program (NTP) explore the effects of compounds with chemical and physical properties similar to diacetyl and acetoin.

2.0 Introduction

Microwave popcorn is a popular snack food in the United States (U.S.) that is consumed by millions of people in their homes, work place, and at many types of recreational events. It was estimated in 2005 that 156 million bags (39 million pounds) of microwave popcorn are consumed each year in the U.S. (Science News, 2005). The consumption of all types of popcorn in 2001 was estimated at one billion pounds per year, of which a large portion was microwaveable. This is equivalent to ~17.5 billion quarts per year or an average of ~ 70 quarts per person per year (Food History, 2001). In order to keep up with consumer demand in the U.S. alone, the microwave popcorn manufacturing industry has to produce over 100 thousand pounds of popcorn per day (assuming a six day work week). One manufacturer alone reported production levels of 150 million bags of microwave and stove-top popcorn in 2006 (Northwest Indiana Times, 2006).

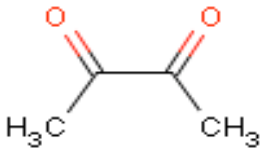
Public Health Concern

Although there is currently no indication that the general public is at risk of developing lung disease from exposure to vapors released from microwaved popcorn, there is growing concern about the risk microwave popcorn producers face. Increased incidences of fixed airway obstruction, including bronchiolitis obliterans, have been recently reported among workers in the microwave popcorn industry. Bronchiolitis obliterans is also called "popcorn worker's lung" or "popcorn packers' lung" and is a rare inflammatory disease that affects the small airways. Its main respiratory symptoms are cough and shortness of breath; the latter may become severe and persistent. The first case of bronchiolitis obliterans in a microwave popcorn packaging worker was seen in 1994, but it was the case reports of eight former workers from the same Missouri plant (Gilster-Mary Lee Corporation) with severe bronchiolitis obliterans syndrome that sparked public interest in May 2000 (Kanwal et al., 2006b [HETA 2000-0401-2991]; Kreiss et al., 2002). Several studies suggest that exposure to volatile organic compounds (VOCs) released from butter flavorings used in production processes is the greatest risk factor. Two prominent VOCs believed to be the major contributors are diacetyl and acetoin (Kreiss et al., 2002). Diacetyl is one of the main components in butter flavoring that gives it its buttery taste and has been identified as a prominent VOC in air samples from microwave popcorn plants (Akpinar-Elci et

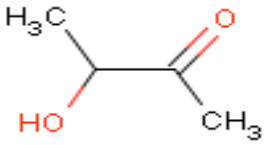
al., 2004; Kanwal, 2003 lett. [HETA 2002-0089]; Kanwal et al., 2004 [HETA 2001-0474-2943], 2006a; Kanwal and Martin, 2003 lett. [HETA 2001-0517]; Parmet and Von Essen, 2002 lett.).

Diacetyl is also naturally found in foods and is used as a synthetic flavoring agent and aroma carrier in butter, caramel, vinegar, dairy products, and coffee. Acetoin is used in the preparation of flavors and essences and found in many of the same foodstuffs as diacetyl (HSDB, 2002, 2005a). The results from toxicological studies published in the open literature and reported in the Hazardous Substance Database (HSDB) for butter flavoring and two of its primary components, diacetyl and acetoin, are reviewed here.

Diacetyl
[431-03-8]



Acetoin
[513-86-0]



2.1 Chemical Identification and Analysis

Diacetyl ($C_4H_6O_2$; mol. wt. = 86.09) is also called:

2,3-Butadione
2,3-Butanedione
2,3-Diketobutane
2,3-Dioxobutane
Biacetyl
Butadiene
Butanedione
Diacetyl (natural)
Dimethyl diketone
Dimethyl glyoxal
Dimethylglyoxal
Glyoxal, dimethyl-
NSC 8750

Source(s): ChemIDplus, 2004a; Registry (1984)
PubChem CID: 650

Acetoin ($C_4H_8O_2$; mol. wt. = 88.10) is also called:

1-Hydroxyethyl methyl ketone
2-Butanone, 3-hydroxy-
2-Hydroxy-3-butanone
2,3-Butanolone
3-Hydroxy-2-butanone
Acethoin

Acetoin (natural)
Acetyl methyl carbinol
Acetylmethylcarbinol
Dimethylketol
γ-Hydroxy-β-oxobutane
Methanol, acetylmethyl-
NSC 7609

Source(s): ChemIDplus (2004b); HSDB (2005a)
PubChem CID: 179

Methods that have been used to analyze diacetyl in foodstuffs (e.g., beer, wine, butter, and butter flavoring) include the National Institute of Occupational Safety and Health (NIOSH) Method #1300, Ketones I: use of gas-chromatography (GC) flame ionization detection (FID) with limit of detection (LOD) at 0.02 mg/sample; NIOSH Method #1301, Ketones II; use of GC-FID with LOD at 0.05 mg/sample; and the Association of Official Agricultural Chemists Method 978.11. Calorimetric methods are also listed as analytical methods for diacetyl analysis (HSDB, 2002). Details of a GC-FID method developed for detecting diacetyl, acetoin, and other ketones in microwave popcorn manufacturing plants are described in Pendergrass (2004 [PMID:14968874]). Sample stability studies using spiked samples collected on Anasorb CMS solid sorbent tubes and stored for seven days at room temperature reported 87-92% (diacetyl) and 63-83% (acetoin) recoveries (Pendergrass, 2004; PMID:14968874). Recoveries up to 95 % were reported for diacetyl samples stored at room temperature for 14 days based on GC-FID analysis (Shah, 2006). GC-FID may also be used to identify VOCs, including diacetyl, in human blood (Houeto et al., 2001).

Thermal desorption with GC-mass spectrometry (GC/MS) has been used to detect diacetyl, acetoin, 2-nanonone, and other VOCs in butter flavorings and air samples from microwave popcorn manufacturing plants (Kanwal and Kullman, 2004 [HETA 2003-0112-2949]; Kanwal et al., 2004 [HETA 2001-0474-2943], 2006b [HETA 2000-0401-2991]; Sahakian et al., 2003 [HETA 2002-0408-2915]). A list of VOCs identified in room air and head space samples from butter flavoring mixtures is given in **Appendix C, Table 1**. Headspace volatiles from unsalted sweet butter heated at 100, 150 or 200 °C for 5 hours were also collected by simultaneous purging and solvent extraction and are included in the table. Analysis by GC-FID, nitrogen-phosphorus, or flame photometric detectors and GC/MS identified 21 aldehydes, 12 fatty acids, 11 ketones (including diacetyl and 2-nonanone; no levels given), 10 nitrogen- and/or sulfur-containing compounds, 7 alkanes, 6 δ-lactones, and 4 furans comprising 85% of total volatiles recovered when heated at 200 °C (Lee et al., 1991).

Diacetyl in foodstuffs can also be indirectly determined by a differential pulse polarographic method; the technique is based on derivatization with *o*-phenylendiamine to yield quinoxaline (Rodrigues et al., 1999; PMID:10552634). In wine, diacetyl can be determined by solid-phase microextraction followed by GC/MS; the detection limit ranged from 0.01 with linearity to 10 µg/mL (Hayasaki and Bartowsky, 1999; PMID:10563940).

Beer samples passed through octadecyl solid-phase extraction column, derivatized with 2,3-diaminonaphthalene and analyzed by high-performance liquid chromatography (HPLC)

identified diacetyl at 2.49-3.53 μg/L. Analysis of headspace from 26 beer samples showed GC compared to HPLC detected diacetyl at a slightly higher, but statistically significant level (McCarthy, 1995).

2.2 Physical-Chemical Properties

Property	Information	Reference(s)
	Diacetyl	
Physical State	yellowish-green liquid	HSDB (2002)
Odor	quinone odor; chlorine-like odor in vapors; rancid butter odor	HSDB (2002)
Boiling Point (°C)	88 @ 760 mm Hg	HSDB (2002)
Melting Point (°C)	-2.4	HSDB (2002)
Flash Point (°C)	7.2 ± 0.0	Registry (1984)*
Vapor Pressure (mm Hg)	56.8 @ 25 °C	HSDB (2002)
Specific Gravity	0.990 @ 15 °C	HSDB (2002)
Water Solubility	200 g/L @ 15 °C	HSDB (2002)
Chemical Solubility	miscible in alcohol, ether; soluble in carbitols; very soluble in acetone	HSDB (2002)
Octanol-water partition coefficient (log P)	-1.34	HSDB (2002)
Bioconcentration Factor (BCF)	1.0 @ 25 °C and pH 1-10	Registry (1984)*
	Acetoin	
Physical State	slightly yellow liquid or crystals	HSDB (2005a)
Odor	buttery	HSDB (2005a)
Boiling Point (°C)	148 @ 760 mm Hg 143	HSDB (2005a); ChemIDplus (2004b)
Melting Point (°C)	15	HSDB (2005a)
Flash Point (°C)	41.1	HSDB (2005a)
Vapor Pressure (mm Hg)	2.7 @ 25 °C	HSDB (2005a)
Specific Gravity	0.9972 g/cm^3 @ 17°C	HSDB (2005a)
Water Solubility	miscible in water	HSDB (2005a)
Chemical Solubility	slightly soluble in alcohol, propylene glycol; soluble in acetone, ether	HSDB (2005a)
Octanol-water partition coefficient (log P)	-0.36	HSDB (2005a)
Bioconcentration Factor (BCF)	0.3	HSDB (2005a)

*Calculated using Advanced Chemistry Development (ACD/Labs) Software Solaris V6.67 [©1994-2004 ACD/Labs]

2.3 Commercial Availability

Butter flavoring is commercially available through the internet from several companies such as Famous Watkins Extracts (best-price.com, 2007); QualityExtracts.com (undated); Alibaba.com (2007); Sysco.com (2006); Country Kitchen SweetArt., Inc (undated); and International Flavors and Fragrances, Inc. (2007). Many of these companies offer both wholesale and retail quantities.

Diacetyl is available in technical, reagent (<99%), analytical (>99%) and food grades. Domestic suppliers found in the Fine Chemicals Database and other sources include Aldrich Chemical Co., Alfa Products, American Tokyo Kasei, Chem Service Inc., Crescent Chemical Co., Eastman Kodak Co., Fischer Chemicals, Fluka Chemical Corp., ICN Biomedicals Corp., International Chemical Group, Jansen Chimica, Lancaster Synthesis, Ltd., Mallinckrodt, Inc., Penta Manufacturing Company, Platz & Bauer, Inc., Sigma Chemical Co., and U.S. Biochemicals Corp. The Environmental Protection Agency (EPA) 1983 TSCA Plant and Production (TSCAPP) database reported other industries that manufactured and processed diacetyl include

Elan Chemical Co. Inc., Fairmount Chemical Co. Inc., Union Carbide Co., Haarmann & Reimer Co., and Givaudan Co. Additional manufacturers and suppliers were found in directories of chemical producers (e.g., Bell Flavors and Fragrances, Inc., CA Aromatics Co.) and included major companies in Europe, the Middle East, and Japan (HSDB, 2002; NTP, 1994).

Companies that produce acetoin include Penta Manufacturing Co. and Sigma Aldrich Fine Chemicals (HSDB, 2005a). Acros Organics also supplies acetoin (85 wt.% in water solution and in 93% practical grade) as well as diacetyl (99%) (ChemExper.com, 2006).

3.0 Production Processes

The use of artificial butter flavorings in microwave popcorn production and the process for preparing the flavoring mixture is described in **Section 7.0**.

Diacetyl is prepared from methyl ethyl ketone by converting it to an isonitroso compound then hydrolyzing with hydrochloric acid to produce diacetyl. It is also produced by oxidation of 2-butanone over a copper catalyst at 300 °C (60% yield), by dehydrogenation of 2,3-butanediol over a copper or silver catalyst in the presence of air, or by acid-catalyzed condensation of 1-hydroxyacetone (obtained by dehydrogenation of 1,2-propanediol) with formaldehyde. Diacetyl is a product of fermentation of glucose via methylacetylcarbinol and of lactic acid bacteria activity during the production of beer (HSDB, 2002). Natural diacetyl can be obtained from starter distillate, a by-product from the manufacture of dairy starter cultures (webexhibits.org, 2006).

Acetoin is prepared from diacetyl by partial reduction with zinc and acid. It is also produced by the action of sorbose bacteria or *Mycoderma aceti* on 2,3-butanediol or by the action of fungi, such as *Aspergillus*, *Penicillium*, or *Mycoderma* on sugar cane juice. Acetoin is a by-product of fermentation and preparation of cream for churning (HSDB, 2005a).

4.0 Production and Import Volumes

The U.S. EPA Inventory Update Rule (IUR) database lists aggregate production volume ranges and companies reporting manufacture for chemicals subject to TSCA IUR reporting requirements. In 1986, 1990, 1994, 1998, and 2002, 10-500,000 pounds of diacetyl production was reported. Citrus & Allied Essences, Ltd. was listed as a manufacturer for 1998 and Elan Chemical Co. for 2002. For acetoin, 10-500,000 pounds of production was reported in 1994 and 1998, and no production reports were listed for 1986, 1990, and 2002. Citrus & Allied Essences, Ltd and Uniroyal Chemcial Co. Inc. were listed as manufacturers for 1998. (USEPA, IUR, 2002)

5.0 Uses

Artificial butter flavoring is a popular ingredient for a variety of commercially available food products and for use in home cooking. Its found in shortenings and butter flavor alternatives (Bunge Oils Inc., 2001; Sweet Celebrations Inc., 2003) and some formulations are used as a flavor enhancing additive in the production of a fat-substitute bakery dough, (Jewell and Seaman, 1994 pat.). Numerous product labels including meat marinades, such as JohnBoy & Billy's Hot & Spicy Grillin' Sauce (Hot Sauce World, undated), low-calorie syrups (Turrisi et al., 1985 pat.), low-calorie simulated cream cheese (Kong-Chan et al., 1991 pat.), and emergency food bars (iPrepare.com, 2006), to name a few, list artificial butter flavoring as an ingredient.

Diacetyl is a key flavoring agent and an aroma carrier in butter flavoring, butter, vinegar, coffee and other foods. It is used as a synthetic flavoring and adjuvant in oleomargarine, candy, baked goods, ice cream, and chewing gum (HSDB, 2002). It is also the primary flavor compound in starter cultures and distillates used in producing cultured butter (webexhibits.org, 2006). Diacetyl is used as a chemical modifier of proteins, combining with arginine residues. It is also used as an electro-stabilizing compound (HSDB, 2002). Other uses for diacetyl include: reactant/starting material in chemical or biochemical reactions; analytical reagent; antimicrobial/preservative; modifier of radiation response for chemical and biological systems; and photoinitiator/ photosensitizer in polymerizations (NTP, 1994).

Acetoin is used as a fragrance carrier and in the preparation of flavors (margarine, butter, milk, yogurt, strawberry) and essences (HSDB, 2005a).

6.0 Environmental Occurrence and Persistence

Diacetyl is a naturally occurring substance found in some foods. It has been reported in butter, caramel, coffee, beer, cocoa, honey, bay and other oils, as well as in aroma components of tobacco smoke (Csiba et al., 1999; HSDB, 2002; O'Neil, 2006; Schmalfuss, 1950). It was identified in dairy products (e.g., cheese, yogurt, milk) by GC/MS analysis (Friedrich and Acree, undated) and was found in oils of finish pine, angelica and lavender, as well as in flower specimens of *polyalthia canangioides boerl* varieties, *angustifolia*, and *fagroea racemosa jack*. Other plants reported to contain diacetyl include *monodora grandiflora benth*, *magnolia tripetale*, *ximenia aegyptiaca*, *petasites fragrans presl*, and various narcissi and tulips. It is also found in plant volatiles, natural aromas of raspberry and strawberry, and in oils of lavandin, reunion geranium, and java citronella (HSDB, 2002; O'Neil, 2006).

Diacetyl photodegrades quickly in the atmosphere (half-life of ~0.7 hr) and is not likely to absorb significantly in soil or sediment or to bioconcentrate in fish (HSDB, 2002; O'Neil, 2006). The photolysis half-life of aqueous-phase diacetyl was 1.0-1.6 hr in a study reporting acetic acid, peroxyacetic acid, and hydrogen peroxide as major and pyruvic acid and methylhydroperoxide as minor photoproducts (Faust et al., 1997).

Acetoin is found in butter, corn, wine, vinegar, honey, cocoa, roasted coffee, and in currant and strawberry aromas. It gradually oxidizes to diacetyl in air and forms a solid dimer on standing or treatment with granulated zinc. The dimer is converted back to the monomer by melting, distilling, or dissolving. Acetion has high mobility if released to soil and is not expected to absorb to suspended solids or sediments or to undergo direct photolysis. A volatilization half-life of 2 days was reported for a model river and 28 days for a model lake; volatilization from dry and moist soil surfaces may also occur. Acetion was reported to have low potential for bioconcentration in aquatic organisms (HSDB, 2005a; O'Neil, 2006).

7.0 Human Exposure

One of the primary concerns for human exposure is that of occupational exposure to VOCs from butter flavoring, specifically diacetyl and acetoin, during the production of microwave popcorn. Increased incidences of fixed airway obstruction have been reported in workers from microwave popcorn manufacturing plants. The incidence is higher among flavoring mixers, packaging

workers, and quality control (QC) personnel. During production butter flavoring mixtures are combined with kernel popcorn in microwave bags by the packaging workers. The flavoring mixtures are prepared by one to three workers (mixers) per shift in mixing rooms that are often kept at 51-54 °C. The mixers measure the butter flavorings (liquid, paste, or powder form) by hand into open containers (e.g., 5-gallon buckets) and pour them into 400- to 800-gallon tanks containing heated soybean oil (54-57 °C). Salt and colorings are also poured by hand into these large tanks. The mixers may spend 1-1.5 hours/day on these procedures. In some plants, the tanks have loose-fitting lids, while in others the tanks have no lids. When lids must be removed to add ingredients, visible clouds of vapors are often released exposing the mixers to volatilized compounds. An evaluation of six microwave popcorn plants reported that only one mixer at one plant regularly used a respirator with organic vapor cartridges (Kanwal et al., 2006a). The heated flavoring mixture is either piped to the packaging lines where it is combined with kernel popcorn in microwaveable bags or to holding tanks then to the packaging lines. In some plants, the packaging process is near the large mixing or holding tanks which increases the packaging workers' risk of exposure to volatiles released from the heated oil/flavoring mixture; increased incidences of obstructive lung disease have been found. QC workers, who microwave up to 100 bags of popcorn per work shift, are also at risk of exposure to oil/flavoring vapors that are released when the popped-corn bag is opened. Some formulations of powdered butter flavorings that are used do not discharge the flavoring chemicals until the popcorn is heated (Kanwal, 2003 lett. [HETA 2002-0089]; Kanwal et al., 2004 [HETA 2001-0474-2943], 2006a). The concentrations (ppm) of five chemicals, including diacetyl and acetoin, found in air samples taken from different work areas in four microwave popcorn plants are shown in **Table 1**. A complete list of chemicals identified, but not quantified, in samples taken from the headspace of butter flavoring mixtures or from room air in manufacturing plants is provided in **Appendix C**, **Table I**. Chemicals in the list that have been tested for pulmonary effects are shown separately in **Table II** of **Appendix C** with a brief description of the reported study results.

According to the 1989 NIOSH National Occupational Exposure Survey, an estimated 3437 workers (1630 females) were exposed to diacetyl (HSDB, 2002). Other occupational exposures to diacetyl and many of the alcohols, aldehydes, esters, fatty acids, ketones, sulfur compounds, and hydrocarbons that microwave popcorn workers have been exposed to include the following:

- Production of flavorings (Hansen and Hoffa, 2006 lett.)
- Manufacturing dairy products or dairy-derived flavoring agents (e.g., Sunesson et al., 2001 [PMID:11354733])
- Food preparation involving cooking with soybean and other seed oils or cooking of meats (Elmore et al., 2001; Schauer et al., 1999a; Schauer et al., 2002a [PMID:11883419])
- Composting biowaste (presumably, municipal solid waste/sewage) (Tolvanen et al., 1998 [PMID:15869986])
- Charcoal production (Greenberg et al., 2006)
- Pig farming (Louhelainen et al., 2001; PMID:11331987).
- Industries involving fermentation with suitable bacteria (e.g., alcoholic beverage and bread production [Annemüller, 1973; Bratovanova, 2001], and
- Carpet laying (diacetyl levels in indoor air were 32 $\mu g/m^3$ and 54 $\mu g/m^3$ after four days of installing latex- or polyurethane-backed carpet, respectively) (NTP, 1994).

Table 1. Air Concentration of Chemicals Identified in Popcorn Manufacturing Plants

Sample Area and Plant Location	Sample Date	Diacetyl[1] ppm	Acetoin ppm	Acetaldehyde ppm	Acetic Acid ppm	Respirable Dust mg/m^3
Mixing Area						
Ridgway, IL	Nov-2002	0.6	0.29	<0.02[2]	0.19	0.48
Sioux City, IA	July-2002	0.08	<0.03	<0.02	<0.02	0.23[3]
Marion, OH	Mar-2004	1.26	1.07	<0.05		0.49
Jasper, MO	Nov-2000	37.8	4.1		5.5	0.37
	July-2003	0.46		<0.04[4]	<0.6[4]	0.19
Processing and/or Packaging						
Ridgway, IL	Nov-2002	0.33	0.18	0.73	0.23	0.42
Sioux City, IA	July-2002	<0.03				0.03 - 0.04[3]
Marion, OH	Mar-2004	<0.03	<0.02	<0.04		0.03 - 0.05
Jasper, MO	Nov-2000	1.69			2.7	0.13
	July-2003	<0.004		<0.04[4]	<0.6[4]	0.03
QC Room						
Ridgway, IL	Nov-2002	0.19	0.15	0.19	0.22	0.62
Sioux City, IA	July-2002	<DL[5]				0.03[3]
Marion, OH	Mar-2004	<0.02	<0.02	0.12		0.03
Jasper, MO	Nov-2000	0.54				NA
	July-2003	<0.004		<0.04[4]	<0.6[4]	

Sources: Sahakian et al. (2003) [Ridgway, IL]; Kanwal et al. (2004) [Sioux City, IA]; Kanwal and Kullman (2004) [Marion, OH]; Kanwal et al. (2006b) [Jasper, MO]

[1] Unless otherwise noted, entries are mean concentrations or a range of concentrations reported if the mean was not given; concentrations reported in mg/m^3 were converted to ppm; [2] means below the quantifiable values are shown as < "quantifiable value"; [3] sampled Sept-2003; [4] sampled Mar-2002; [5] <DL = below detectable limit

Sources of diacetyl in ambient air to which the general population may be exposed include:

- Exhaust emissions from combustion of petroleum-derived fuels in diesel-, gasoline-, and jet-fuel-powered engines (Schauer et al., 1999b, 2002b [PMID:11944666]; Spicer et al., 1992).
- Fine airborne particulate matter such as that sampled in a California roadway tunnel (Rao et al., 2001; PMID:11417634).
- Cigarette smoke (Fujioka and Shibamoto, 2006; PMID:16463255)
- As a secondary air pollutant resulting from photooxidation of the common gasoline aromatics toluene, xylenes, and ethylbenzene and of methyl-substituted aromatic hydrocarbons reacting with nitrogen oxides (NTP, 1994)
- Volatilization of diacetyl-containing aqueous and solid livestock wastes (NTP, 1994)
- Moldy buildings (Wilkins et al., 1997)

Diacetyl and acetoin are also formed endogenously in humans from decarboxylation of pyruvate (HSDB, 2005a).

8.0 Regulatory Status

In the United States, diacetyl is regulated by the FDA as a substance directly added to human food; it is generally recognized as safe (GRAS) [21 CFR 184.1278] (HSDB, 2002). Acetoin is also regulated by the FDA as a synthetic flavoring substance/adjuvant for human consumption [21 CFR 182.60] and for animal drugs, feeds, and related products [21 CFR 582.60]; it is also

GRAS (HSDB, 2005a). There are no specific occupational exposure limits for diacetyl or acetoin.

9.0 Toxicological Data

9.1 General Toxicology

Toxicology information reviewed here focuses on inhalation studies of butter flavoring VOCs, diacetyl, and acetoin and their effects on the lungs and respiratory tract. Additional toxicological data that were available from reviews (e.g., an NTP chemical background document [http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/431-03-8.pdf] prepared for the National Cancer Institute in 1994 and profiles from the Hazardous Substances Data Bank [HSDB]) are briefly summarized.

9.1.1 Human Data

In 1994, one case of bronchiolitis obliterans was observed in a packaging worker at a Jasper Missouri microwave popcorn manufacturing plant (Gilster-Mary Lee Corporation) (Kanwal et al., 2006b [HETA 2000-0401-2991]; Kreiss et al., 2002). Additional incidence of bronchiolitis obliterans or obstructive lung disease in other microwave popcorn manufacturing plants, including at least three deaths, have since been reported (Akpinar-Elci et al., 2004; Kanwal, 2003 lett. [HETA 2002-0089]; Kanwal and Martin, 2003 lett. [HETA 2001-0517]; Kreiss et al., 2002; Parmet and Von Essen, 2002 lett.). These include:

- Eight former workers from the Jasper Missouri plant were diagnosed with bronchiolitis obliterans (Kreiss et al., 2002; see Kanwal et al., 2006b [HETA 2000-0401-2991]); some are candidates for lung transplants:
  - four worked in the butter flavor mixing room, and
  - four worked in the packaging areas.
- Three workers at B.K. Heuermann Popcorn, Inc. (Phillips, Nebraska) had mild/borderline airway obstruction. One case was linked to exposure to butter flavorings; exposure of the other two cases to butter flavoring mixtures was unknown (Kanwal and Martin, 2003 lett. [HETA 2001-0517]).
- A butter flavoring mixer at American Pop Corn Company (Sioux City, Iowa) was diagnosed with fixed obstructive lung disease consistent with bronchiolitis obliterans (Kanwal et al., 2004 [HETA 2001-0474-2943]).
- A butter flavoring mixer at ConAgra Snack Foods (Marion, Ohio) was diagnosed with severe fixed obstructive lung disease consistent with bronchiolitis obliterans.
  - 3 of 12 slurry room workers had obstruction of their airways on spirometry and normal diffusing capacity consistent with bronchiolitis obliterans.
  - 5 packaging area workers had fixed pulmonary obstruction and normal diffusing capacity.
  - 2 of 11 Quality Assurance workers had abnormal spirometry: one had obstruction and normal diffusing capacity; the other had restriction (Kanwal and Kullman, 2004 [HETA 2003-0112-2949]).
- At the Agrilink Foods Popcorn Plant (Ridgway, Illinois), 10 of 41 workers were suspected of having bronchiolitis obliterans [Note: The plant's popcorn packaging operations closed January 30, 2003] (Sahakian et al., 2003 [HETA 2002-0408-2915].

Diacetyl was the predominant compound found in the artificial butter flavoring and the room air of the Jasper Missouri plant. Workers in the mixing room and packaging area (production areas) were exposed to diacetyl concentrations that were ~800x and 15x, respectively, the mean concentrations for the office, warehouse, and outside areas. Compared to workers in these areas, production area workers had significantly higher rates of shortness of breath on exertion, breathing problems, a combination of respiratory symptoms, unusual fatigue, other systemic symptoms, and rashes or other skin problems. As the cumulative exposure to diacetyl increased, the incidence of airway obstruction and abnormal results on spirometry (i.e., airway obstruction or low forced vital capacity) increased and the average one second forced expiratory volume decreased (Kreiss et al., 2002). High-resolution computed tomography (HRCT) showed significant bronchial wall thickening and mosaic shrinking with air trapping. Lung biopsies from three fatal cases showed rare granulomas, emphysema, pneumothoraces, and/or other symptoms of constrictive bronchiolitis. Airways obstruction was not improved in any of the cases by treatment with oral corticosteroids. Bronchiolitis obliterans was characterized as having fixed obstruction, normal chest radiographs, and bronchiectasis and air trapping on HRCT (Akpinar-Elci et al., 2004).

Although diacetyl is thought to be the primary contributor to respiratory disease in popcorn manufacturing, workers in production areas were also exposed to the highest concentrations of ketones, other VOCs, and respirable dust (Kreiss et al., 2002). Therefore, diacetyl may not be the only factor contributing to bronchiolitis obliterans; e.g., tannins have also been proposed as a causal factor (Kreiss et al., 2002; Taubert et al., 2002 lett.).

In a more recent study, risk for bronchiolitis obliterans was assessed at six microwave popcorn plants, using the Missouri facility as an index plant. An eight-hour time-weighted average concentration of diacetyl was used as an indicator of exposure to butter-flavoring VOCs. Mixers (those having mixed butter flavorings and oil for at least one day) had higher incidences of all respiratory symptoms (shortness of breath, chronic cough, and wheezing) compared to those who had never worked in the mixing room; mixers having worked >12 months had higher incidence of all respiratory symptoms *and* airways obstruction compared to those with less experience. Packaging workers in plants where butter flavoring mixing tanks were not adequately separated from the packaging areas also had higher frequencies of all respiratory symptoms and airways obstruction. Additionally, five of six QC workers in the index plant had airways obstruction and the highest mean diacetyl air concentration (0.6 ppm) was found in the QC laboratory (Kanwal et al., 2006a).

Workers exposed to butter flavoring vapors have also reported eye irritation (chemical burns), skin irritation, and nasal irritation (Kanwal, 2003 lett. [HETA 2002-0089]; Kanwal and Martin, 2003 lett. [HETA 2001-0517]). Patch testing and maximization testing with diacetyl produced no irritation or sensitization, respectively, in volunteers (NTP, 1994). Tests with acetoin also resulted in no irritation or sensitization reactions (Opdyke, 1979).

Other industries that have reported incidence of bronchiolitis obliterans in their staff include two workers in a mixing facility of the baking industry (affected within five months of working), workers in flavoring manufacturing plants, and a snack food manufacturing worker who was using flavorings and spices (Akpinar-Elci et al., 2004; Kreiss et al., 2002).

9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

The metabolic pathway for diacetyl reduction to acetoin and 2,3-butanediol is shown below in **Figure 1**. Results from metabolism studies with rat liver preparations and studies of rats exposed *in vivo* are summarized following the figure.

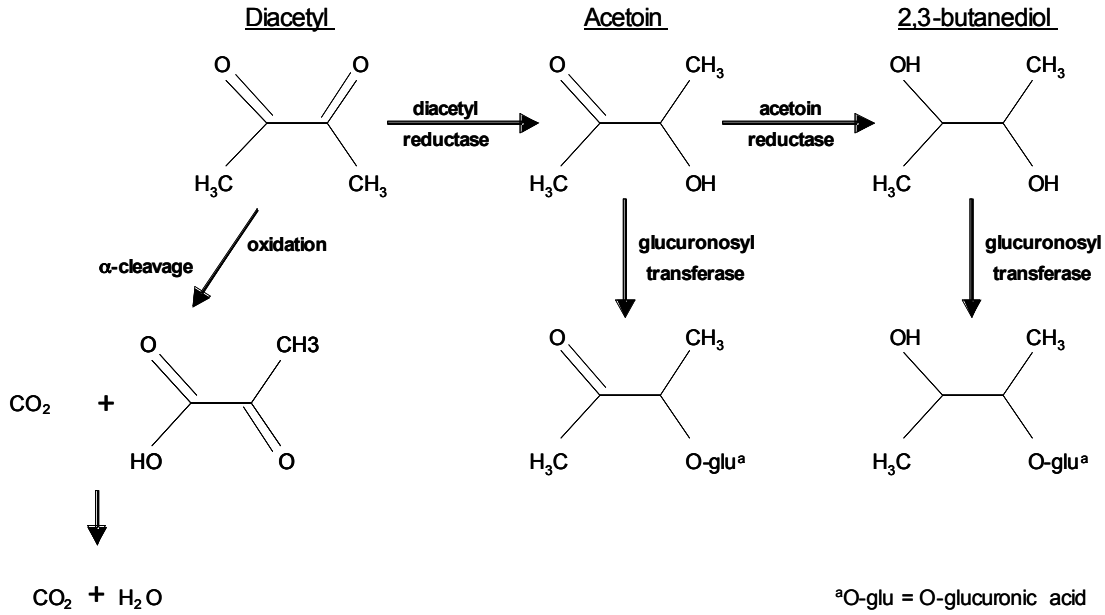


Figure 1. Diacetyl/acetoin metabolism

It is anticipated that humans will metabolize aliphatic acyclic methyl ketones principally by oxidation of the terminal methyl group at low levels of exposure. At higher levels, reduction to the diol and subsequent conjugation with glucuronic acid is a competing detoxification pathway. Other aliphatic acyclic alpha-diketones and alpha-hydroxyketones are reduced, conjugated with glucuronic acid and excreted.

Diacetyl

A single oral dose of diacetyl (430 mg/kg bw) was metabolized by reduction to acetoin in male Wistar albino rats. Acetoin was found in high concentrations in major organs one hour after dosing. 2,3-Butanediol, the reduction product of acetoin, was detected in the liver, kidney and brain. Rat liver homogenate rapidly (10 minute incubation) converted 10 nmol (9 x 10-4 mg) diacetyl to 3.7 nmol (3 x 10-4 mg) acetoin and 6.3 nmol (6 x 10-4 mg) butane-2,3-diol (Otsuka et al., 1996).

When administered to male Fischer 344 rats via intragastric gavage, a single dose of radiolabeled [^{14}C]-diacetyl (1.58, 15.8, or 158 mg/kg [0.0184, 0.184, or 1.84 mmol/kg]) resulted in excretion of 82.0, 72.7, and 54.3% of the administered doses, respectively, as carbon dioxide at 72 hours. In urine, the excreted amounts were 6.86, 15.7, and 34.1%, respectively. At the high dose, elimination via volatile organics in breath and feces was insignificant (maximums of 0.8 and 2.25%, respectively). In the carcass and tissues, 6-7% of the dose was recovered. At all tested levels, diacetyl was rapidly metabolized and excreted; excretion of radioactivity in urine, feces, and expired breath accounted for 86-87% of the total dose recovered in 24 hours (RTI, 1997).

In a rat liver preparation, diacetyl and ampicillin were formed as metabolites from the hydrolyzation of KB-1585, an ester of ampicillin; diacetyl was then metabolized to 2,3-butanediol via acetoin. In normal rat liver mitochondria, diacetyl uncoupled oxidative phosphorylation, totally eliminated respiratory control with substrates and partially eliminated it with succinate (HSDB, 2002). Metabolic interconversions between diacetyl, acetoin and 2,3-butanediol was observed with rat liver extracts (NTP, 1994). All three compounds were also acetaldehyde metabolites in perfused liver. Diacetyl reduction to acetoin required NADH or NADPH; acetoin reduction to 2,3-butanediol required NADH (Otsuka et al., 1996; PMID:8882713).

Acetoin

Metabolism of acetoin *in vivo* is mainly by oxidation at low concentrations and by reduction to 2,3-butanediol at high concentrations. In a 24-hour period, 1 g of rat liver was estimated to oxidize 86 µg (1 µmol) acetoin. In rat and rabbit liver extracts, >95% of radiolabeled [2,3-^{14}C]-acetoin was detected as a mixture of 2,3-butanediol stereoisomers. When acetoin (doses not provided) was i.p. injected into albino rats, ^{12}C-carbon dioxide was found in expired air (average of 15% during 12 hours). When acetoin was administered orally (3-4% solution) or subcutaneously (20% solution) to rats, no diacetyl and very little acetoin were detected in the urine; 2,3-butanediol was the major excretion product (HSDB, 2005a). In rabbits orally given acetoin and in rabbit liver homogenate incubated with acetoin (doses not provided), acetylation was increased. In male guinea pigs, acetoin was an intermediary metabolite in the reduction of methyl ethyl ketone to 2,3-butanediol (Opdyke, 1979).

9.1.3 Acute Exposure

Acute toxicity values for diacetyl and acetoin are presented in **Table 2**. Diacetyl was observed to be a severe skin and eye irritant in rabbits (NTP, 1994). Acetoin was a moderate irritant on intact and abraded skin of rabbits (Opdyke, 1979).

Table 2. Acute Toxicity Values for Some Artificial Butter Flavoring Components

Route	Species (sex and strain)	LD_{50}/LC_{50}	Reference(s)
Diacetyl [431-03-8]			
inh	rat (M, F; Wistar)	$2.25 < LC_{50} < 5.2$ mg/L [4-hr] ($2250 < LC_{50} < 5200$ mg/m^3; $639 < LC_{50} < 1477$ ppm)	BASF (1993)
i.p.	mouse (sex and strain n.p.)	LD_{50} = 249 mg/kg (2.89 mmol/kg)	NTP (1994)
	rat (sex and strain n.p.)	LD_{50} = 400-650 mg/kg (4.65-7.55 mol/kg)	ChemIDplus (2004a); NTP (1994)
oral	mouse (sex and strain n.p.)	LD_{50} = 250 mg/kg (2.90 mmol/kg)	NTP (1994)
	rat (sex and strain n.p.)	LD_{50} = 1580 mg/kg (18.35 mmol/kg)	ChemIDplus (2004a); NTP (1994)
	guinea pig (sex and strain n.p.)	LD_{50} = 990 mg/kg (11.50 mmol/kg)	ChemIDplus (2004a); NTP (1994)
dermal	rabbit (sex and strain n.p.)	LD_{50} > 5000 mg/kg (58.08 mmol/kg)	ChemIDplus (2004a); NTP (1994)
Acetoin [513-86-0]			
oral	rat (sex and strain n.p.)	LD_{50} > 5000 mg/kg (56.75 mmol/kg)	ChemIDplus (2004b)
dermal	rabbit (sex and strain n.p.)	LD_{50} > 5000 mg/kg (56.75 mmol/kg)	

Abbreviations: F = female(s); hr = hour(s); inh = inhalation; i.p. = intraperitoneal; LC_{50} = concentration lethal to 50% of test animals; LD_{50} = lethal dose for 50% of test animals; M = male(s); n.p. = not provided

Details of the following ***inhalation*** studies are provided in **Table 3**.

Artificial Butter Flavoring

Male rats exposed to vapors from artificial butter flavoring (average diacetyl concentrations: 203, 285, 352 [constant], or 371 [pulsed] ppm; range 72-940 ppm) for six hours exhibited necrosis of nasal and airway epithelium. At levels of 285-371 ppm diacetyl, necrotizing bronchitis was observed in the lung; at 203-371 ppm diacetyl, necrosuppurative rhinitis and inflammation were seen at all nasal levels (Hubbs et al., 2002).

Diacetyl

In male C57BL/6 mice inhalation of 200 or 400 ppm diacetyl six hours per day for five days caused death and acute necrotizing rhinitis, laryngitis and bronchitis (proximal large bronchi) in the 400 ppm group. A few deaths and acute necrotizing rhinitis and erosive or necrotizing laryngitis were observed at 200 ppm but lung or bronchiolar lesions were not. One hour exposure per day for four weeks (100, 200, 400 ppm) resulted in chronic bronchitis, laryngitis, and rhinitis after two and four weeks. The response was concentration related ranging from minimal to moderate. Two of five mice given 400 mg/kg diacetyl by oropharyngeal aspiration died two days after aspiration. Foci of fibrosis without inflammation were observed at the junction of the terminal bronchiole and alveolar duct in the three surviving mice. Similar lesions with mild inflammation were noted in one of five mice treated with 200 mg/kg (Morgan et al., 2006 abstr.).

Inhalation of diacetyl (99, 198, or 295 ppm [349, 697, or 1039 mg/m^3]) for six hours also produced significant necrosis of nasal epithelium at ≥198 ppm and significant necrosis of tracheal epithelium at the high dose in male rats. No significant effects in the lung were reported (Hubbs et al., 2004 abstr.).

Table 3. Acute Inhalation Exposure to Artificial Butter Flavoring and Diacetyl

Species, Strain, and Age, Number, and Sex of Animals	Chemical Form and Purity	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Artificial Butter Flavoring				
Rats, Sprague-Dawley (Hla:[SD]CVF), age n.p., 19M total (6, 4, 6, 3, respectively, with dose [see column 3])	Butter flavoring vapors (butter heated to 50 °C for 10 min), purity N/A	inh (via whole-body exposure chamber); 203, 285, 352 (constant), or 371 (pulsed) ppm [average diacetyl concentrations]; 6 hours; necropsied 1 day later	Major peaks of vapors (headspace): diacetyl, acetic acid, butyric acid, acetoin, acetoin dimers, 2-nonanone, and δ–alkyl lactones At the mid-constant and high-pulsed doses, one rat each died after exposure. Pulmonary findings: At the mid and both high doses, rats had inflammatory and/or necrotizing changes in the airways, consisting of multifocal to multifocal and coalescent distribution with moderate to significant severity; the main morphologic change was necrotizing bronchitis with decreasing severity of necrosis in smaller airways. Constant and pulsed exposures resulted in similar lesions in the mainstem bronchus, but necrosis was confined to the mainstem bronchus in constant-exposed rats and to the mainstem bronchus and midsize bronchioles in pulsed-exposed rats. Airway and bronchiolocentric changes were increased in mid- and both high-dosed rats compared to controls. BAL PMNs were significantly increased in mid- and high-constant rats. BAL albumin concentration and macrophage chemiluminescence were significantly increased in both high-dose groups. Ultrastructure studies mainly showed necrosis of bronchial epithelium; with the mid- and high-constant doses, bronchial injury extended beneath the basement membrane and had edema of the lamina propria. Nasal findings: Suppurative inflammation and necrosis of the epithelium lining nasal passageways were observed in all exposed rats; necrosis sometimes extended beneath epithelial basement membrane. Coalescing foci of necrosuppurative rhinitis was seen in respiratory, transitional, and olfactory epithelium. Mid-dose rats had hypercellularity with mostly PMN in the nasal lavage fluid.	Hubbs et al. (2002)

Table 3. Acute Inhalation Exposure to Artificial Butter Flavoring and Diacetyl (Continued)

Species, Strain, and Age, Number, and Sex of Animals	Chemical Form and Purity	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Diacetyl				
Rats, Sprague-Dawley, age and number n.p., M	diacetyl, purity n.p.	inh (via whole-body exposure chamber); 0, 99.3±0.07, 198.4±0.10, or 294.6±0.20 ppm for 6 hours; killed the next day	Significant effects in the lung were not observed. At the mid and high doses, rats developed necrosis of nasal epithelium with associated neutrophilic inflammation. At the high dose, necrosis of tracheal epithelium with associated neutrophilic inflammation was also seen. Tracheal changes included the following: SEM: multifocal denuding of basement membrane with cell swelling, loss of microvili, and loss of ciliated cells in epithelium TEM: epithelial necrosis, denuded basement membrane, and elongation of epithelial cells near foci of exposed basement membrane	Hubbs et al. (2004 abstr.)
Rats, Wistar (SPF Wistar/Chbb:THOM), 8- to 9-wk-old, 5M and 5F per dose group	Diacetyl FCC vapor, 99.1% pure	inh (via whole-body exposure chamber); 2.25, 5.2, and 23.9 mg/L for 4 hours; observed for 14 days	At the low dose, acute signs included eyelid closure, restlessness, apathy, squatting posture, and ruffled furs in all rats; some animals also showed abdominal and dragging respiration, respiratory sounds, and reduced general state. By day 5, clinical signs were no longer observed. At the mid and high doses, all rats died. Necropsy findings: general congestion at the mid and high dose; focal hyperemia of the lungs and empty gastrointestinal tract in mid-dose rats; focal atelectasis (lobes) and bloody edema of lungs, edema and intensified hydrothorax of the bronchi in high-dose rats Microscopic findings: peripheral swelling of liver hepatocytes and moderate emphysema and focal hyperemia in lungs of mid-dose rats; extensive hyperemia in lungs, necrosis in proximal part of kidney tubulus, centrolobular swelling of liver hepatocytes in high-dose rats $2.25 < LC_{50} < 5.2$ mg/L	BASF (1993)

Abbreviations: BAL = bronchoalveolar lavage; F = female(s); M = male(s); min = minute(s); N/A = not applicable; n.p. = not provided; PMN = polymorphonuclear leukocyte; wk = week(s)

In male and female Wistar rats, inhalation of diacetyl FCC (2.25, 5.2, and 23.9 mg/L [639.0, 1477, or 6788 ppm]) for four hours resulted in deaths at the mid and high doses. Necropsy showed general congestion in dead rats, focal hyperemia of the lungs and empty gastrointestinal tract in mid-dose animals, and atelectasis and bloody edema of the lungs, bronchial edema, and intensified hydrothorax in high-dose rats. Additionally, histopathological examination revealed moderate emphysema and focal hyperemia of the lungs as well as peripheral swelling of hepatocytes at the mid dose, and widespread hyperemia of the lung, necrosis in the proximal tubules of the kidney, and centrilobular swelling of hepatocytes at the high dose (BASF, 1993).

In *in vitro* assays, diacetyl (1, 3, or 10 mM [86, 258, or 861 µg/mL]) caused contraction and relaxation in perfused guinea pig trachea preparations and damage of the epithelium. At the high dose, diacetyl completely inhibited responses of the perfused trachea to methacholine but had no effect on reactivity to terbutaline or potassium chloride (Fedan et al., 2006).

Acetoin
No acute inhalation studies were available.

9.1.4 Short-term and Subchronic Exposure

Diacetyl
No short-term or subchronic inhalation studies were available.

Daily oral administration of diacetyl (10, 30, 90, or 540 mg/kg [0.12, 0.35, 1.0, or 6.27 mmol/kg]) for 90 days to rats produced decreased weight gain, increased water consumption, anemia, increased leukocyte count, and increased weights of the liver, kidney, adrenal gland, and pituitary gland. Necrosis in the stomach was also observed [NOEL = 90 mg/kg] (HSDB, 2002).

Acetoin
No short-term or subchronic inhalation studies were available.

When male and female CFE rats were given acetoin (750, 3000, or 12,000 mg/kg [85, 330, or 1300 mg/kg bw/day) in the drinking water for 13 weeks, no effects on condition or appearance and no deaths occurred. At the high dose, male body weights were significantly decreased from week 5 and relative liver weight was statistically significantly increased at weeks 2, 6, and 13; females showed these effects after 13 weeks. (The effect on the liver may have been due to an increased metabolic load from the high dose.) A small but statistically significant decrease in hemoglobin concentration and erythrocyte counts was also observed at the high dose in both sexes. Urinalysis, blood chemistry, and histopathology revealed no other adverse effects [NOEL = 3000 ppm, 330 mg/kg bw/day] (HSDB, 2005a).

9.1.5 Chronic Exposure

No data were available.

9.1.6 Synergistic/Antagonistic Effects

When small amounts of 30% acetoin solution (doses not provided) were. injected i.p into rats to cause loss of righting reflex or respiratory failure, blood acetoin concentrations ranged from 227-251 mg percent (average 235 mg) and from 742-770 mg percent (average 754 mg), respectively.

When ethanol was injected i.p into rats, alcohol levels ranged from 288-312 mg and 900-952 mg, respectively. When acetoin and ethanol administration was combined, the concentrations of both chemicals in blood were additive (HSDB, 2005a).

9.1.7 Cytotoxicity

Diacetyl (0.001, 0.1, or 1 mM [0.086, 8.6, or 86 µg/mL]) inhibited cell growth in ascites sarcoma cells by 37% at the mid dose and by 100% at the high dose (HSDB, 2002).

No data were available for acetoin.

9.2 Reproductive and Teratological Effects

When given via oral intubation to pregnant mice for ten days, diacetyl (1.6 g starter distillate/kg) had no effects on maternal or fetal survival or nidation. There were also no statistically significant changes in the number of fetal abnormalities compared to controls. Tests in hamsters and rats gave similar results (HSDB, 2002).

No data were available for acetoin.

9.3 Carcinogenicity

When given i.p. to mice once weekly for 24 weeks, diacetyl (1.70 or 8.40 mg/kg [0.0197 or 0.0976 mmol/kg]) did not induce any lung tumors (HSDB, 2002; NTP, 1994). Acetoin (total doses of 12.0 or 60.0 g/kg [136 or 681 mmol/kg] given i.p. 3x/week for 6-7 weeks) also showed no carcinogenic activity (Opdyke, 1979).

9.4 Initiation/Promotion Studies

No data were available.

9.5 Anticarcinogenicity

No data were available.

9.6 Genotoxicity

Diacetyl

In several bacterial assays, diacetyl generally showed mutagenic activity in *Salmonella typhimurium* strains TA100, 102, and 104 with and without metabolic activation but none against strain TA98. Conflicting results were obtained in *Escherichia coli* strain WP2 uvra, but nonmutagenicity was demonstrated in the SOS-chromotest using *E. coli* PQ37. Diacetyl was also negative in a micronucleus test using mouse bone marrow cells (CCRIS, 1995; NTP, 1994). Diacetyl induced sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) AUXB1 cells and unscheduled DNA synthesis in various organs of laboratory animals, such as the rat stomach mucosa (HSDB, 2002; NTP, 1994).

Acetoin

Acetoin (up to 4500 mg/plate [51.08 mmol/plate]) was generally nonmutagenic in bacteria *in vitro* (HSDB, 2005a).

9.7 Cogenotoxicity

Diacetyl induced mitotic chromosome loss in *Saccharomyces cerevisiae* only in the presence of propionitrile (NTP, 1994).

9.8 Antigenotoxicity

In CHO AUXB1 cells, bisulfite significantly reduced the frequency of SCEs and proportion of endoreduplicated cells when diacetyl was administered. Sodium sulfite almost completely inactivated the mutagenicity of diacetyl in *S. typhimurium* strain TA100. The reaction of diacetyl with heterocyclic amines also significantly suppressed the mutagenicity in the bacterial strain (NTP, 1994).

9.9 Immunotoxicity

Workers exposed to butter flavoring vapors have also reported eye (chemical burns), skin, and nasal irritations. Patch testing and maximization testing with diacetyl produced no irritation or sensitization, respectively, in volunteers (NTP, 1994). Tests with acetoin also resulted in no irritation or sensitization reaction (Opdyke, 1979).

9.10 Other Data

Effects on Enzymes

When administered to rats via gastric intubation, diacetyl (300 or 1500 mg/kg bw [3.48 or 17.42 mmol/kg bw]) produced increases in ornithine decarboxylase activity and DNA synthesis in the pyloric mucosa (HSDB, 2002; NTP, 1994). Diacetyl has activating and deactivating effects on several enzymes and metabolic processes, including inactivating estradiol 17β-dehydrogenase in human placenta under ultraviolet light (NTP, 1994). Diacetyl reduced the internalization of the lysosomal enzyme α-*L*-iduronidase into human diploid fibroblasts by 50% without affecting enzyme activity and reduced binding to the fibroblast membranes by 90%; a similar reduction was also seen in membranes from rat chondrosarcomas (Rome and Miller, 1980).

Possible Mechanism for Lung Damage by Diacetyl

Diacetyl may induce lung damage by oxidative stress. Reduction potentials for diacetyl and its iminium derivatives were found to be in the range favorable for catalytic electron transfer *in vivo*, which can cause oxidative stress via reaction oxygen species as a result of redox cycling (Kovacic and Cooksy, 2005; PMID:15654607). *In vivo*, diacetyl may be involved in redox cycling with acetoin and with imino compounds formed by condensation with ammonia or the free amino groups of proteins (Yaylayan et al., 2005; PMID:16037220). Lung damage caused by ozone has also been suggested to be due to the formation of reactive free radicals (HSDB, 2005b).

10.0 Structure-Activity Relationships

Genetic and carcinogenic effects for several analogs, such as methylglyoxal and acetaldehyde, are included in the background document for diacetyl provided by the NTP (http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/431-03-8.pdf).

11.0 Online Databases and Secondary References

11.1 Online Databases

National Library of Medicine Databases (TOXNET)
CCRIS
ChemIDplus
DART
GENETOX
HSDB
IRIS

STN International Files

AGRICOLA	IPA
BIOSIS	MEDLINE
BIOTECHNO	NIOSHTIC
CABA	NTIS
CANCERLIT	Registry
EMBASE	RTECS
ESBIOBASE	TOXCENTER

TOXCENTER includes toxicology data from the following files:

Aneuploidy	ANEUPL*
BIOSIS Previews® (1969-present)	BIOSIS*
CAplus (1907-present)	CAplus
International Labour Office	CIS*
Toxicology Research Projects	CRISP*
Development and Reproductive Toxicology	DART®*
Environmental Mutagen Information Center File	EMIC*
Epidemiology Information System	EPIDEM*
Environmental Teratology Information Center File	ETIC*
Federal Research in Progress	FEDRIP*
Health Aspects of Pesticides Abstract Bulletin	HAPAB
Hazardous Materials Technical Center	HMTC*
International Pharmaceutical Abstracts (1970-present)	IPA*
MEDLINE (1951-present)	MEDLINE
Pesticides Abstracts	PESTAB*
Poisonous Plants Bibliography	PPBIB*
Swedish National Chemicals Inspectorate	RISKLINE
Toxic Substances Control Act Test Submissions	TSCATS*

*These are also in TOXLINE. Missing are TOXBIB, NIOSHTIC®, NTIS.

National Archives and Records Administration
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In-House Databases
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Acknowledgements

Support to the National Toxicology Program for the preparation of Chemical Information Review Document for Artificial Butter Flavoring was provided by Integrated Laboratory Systems, Inc., through NIEHS Contract Number N01-ES-35515. Contributors included: Scott A. Masten, Ph.D. (Project Officer, NIEHS); and, from ILS, Inc., Marcus A. Jackson, B.A. (Principal Investigator), Bonnie L. Carson, M.S., Claudine A. Gregorio, M.A., Yvonne H. Straley, B.S., Pamela Y. MaGee, B.S., and Sherry D. Blue, A.A.

Appendix A: Units and Abbreviations

ºC = degrees Celsius
µg/L = microgram(s) per liter
µg/m^3 = microgram(s) per cubic meter
µg/mL = microgram(s) per milliliter
µM = micromolar
bw = body weight
CCRIS = Chemical Carcinogenesis Research Information System
CHO = Chinese hamster ovary
DNA = deoxyribonucleic acid
EPA = Environmental Protection Agency
F = female(s)
FDA = Food and Drug Administration
FID = flame ionization detection
g = gram(s)
g/kg = gram(s) per kilogram
g/mL = gram(s) per milliliter
GC = gas chromatography
GRAS = generally recognized as safe
hr = hour(s)
HRCT = high-resolution computed tomography
HSDB = Hazardous Substances Data Bank
i.p. = intraperitoneal(ly)
kg = kilogram(s)
lb = pound(s)
LC = liquid chromatography
LC_{50} = lethal concentration for 50% of test animals
LD_{50} = lethal dose for 50% of test animals
LOD = limit of detection
M = male(s)
mg/kg = milligram(s) per kilogram
mg/L = milligram(s) per liter
mg/m^3 = milligram(s) per cubic meter
mg/mL = milligram(s) per milliliter
mm = millimeter(s)
mM = millimolar
mmol = millimole(s)
mmol/kg = millimoles per kilogram
MMWR = Morbidity and Mortality Weekly Report
mol = mole(s)
mol. wt. = molecular weight
NIOSH = National Institute for Occupational Safety and Health
NOEL = no observable effect level
n.p. = not provided
NTP = National Toxicology Program
OSHA = Occupational Safety and Health Administration

PEL = permissible exposure limit
ppm = parts per million
QC = quality control
SCE = sister chromatid exchange
TSCA = Toxic Substances Control Act
TSCAPP = TSCA Plant and Production
UFCW = United Food and Commercial Workers
VOC = volatile organic compound
wk = week(s)

Appendix B: Description of Search Strategy and Results

An initial search of the usual biomedical databases (MEDLINE, CABA, AGRICOLA, EMBASE, BIOTECHNO, ESBIOBASE, BIOSIS, IPA, and TOXCENTER) was conducted using only the CAS Registry Numbers to represent numerous compounds found in butter or butter flavoring. Keywords for the concepts lung injury, cancer, and association with flavoring butter, margarine, or popcorn were combined with the aggregate answer number for the compounds. A later search for diacetyl and acetoin was restricted to CAPLUS. Their CAS numbers were combined with concepts for adverse effects, industrial hygiene, or occurrence in the environment.

Brief searches for certain other butter flavoring volatiles or other compounds found in room air at two facilities were done in Google Scholar, PubMed, and the TOXNET databases, especially ChemIDplus, HSDB, and TOXLINE. These compounds searched were generally aldehydes, carboxylic acids, and others suspected of having possible lung toxicity because of their chemically reactive nature. Searches were also done to identify VOCs from heated soybean oil, heated butter, and heated popcorn kernels in the absence of added flavoring. Heated butter is of interest because natural butter flavorings would be expected to have many more volatiles than just the few compounds selected for manufacturing artificial flavors. Searches confirmed that hydrogen sulfide is reported by several references to be a major volatile from microwave popping of unflavored popcorn kernels or other heating of corn, but no original source was found to confirm that hydrogen sulfide exposure may lead to bronchiolitis obliterans as stated by Boswell and McCunney (1995).

Appendix C. Volatile Organic Compounds (VOCs) in Popcorn Manufacturing

Table I. VOCs identified in head space samples from butter flavoring mixtures and/or air samples from three microwave popcorn plants [a]

Chemical Name	CASRN	PubChem CID	Dry [b] popcorn	Sweet [c] Butter at: (˚C)	Butter Flavor [d] Ridgway (Liq. or Paste)	QC Room Ridgway [e]	Mixing Room Ridgway [e]	Mixing Room Sioux City [f] (Liq. or Paste)	Mixing Room Sioux City (Powder)	Mixing Room Jasper [g]
Major Constituents Identified in Butter Flavoring and Found in Room Air										
Diacetyl; 2,3-Butanedione [h]	431-03-8	650	x	100	**Major**	**Major**	**Major**	**Major**	Minor	**Major**
Acetic acid	64-19-7	176	x		**Major**	**Major**	**Major**	**Major**	Minor	**Major**
2-Butanone; Methyl ethyl ketone	78-93-3	6569	x		**Major**	**Major**	**Major**	**Major**	**Major**	**Major**
Ethanol	64-17-5	702	x		**Major**	**Major**	**Major**	**Major**	**Major**	NL
Limonene d,l-Limonene; Dipentene (R)-(+)-Limonene; d-Limonene (S)-(-)-Limonene; l-Limonene	138-86-3 5989-27-5 5989-54-8	22311 440917 439250		100	**Major**	**Major**	**Major**	**Major**	**Major**	**Major** (QC room)
Butyric acid	107-92-6	264		100	**Major**	**Major**	Minor	**Major**	Minor	NL
Ethyl butyrate	105-54-5	7929	x		**Major**	**Major**	**Major**	Minor	Minor	NL
Ethyl acetate [with 2-ethyl-2-methyloxirane for Ridgway]	141-78-6	8857			**Major**	**Major**	**Major**	Minor	?	NL
Acetonitrile*	75-05-8	6342			**Major**	**Major**	**Major**	?	Minor	NL
Decane	124-18-5	15600	x	150	**Major**	**Major**	**Major**	*Minor*	?	NL
3-Methylacetoin; 3-Hydroxy-3-methyl-2-butanone	115-22-0	8261			**Major**	Minor	Minor	Minor	*Minor*	NL
Acetoin; 3-Hydroxy-2-butanone	513-86-0	179	x		**Major**	**Major**	**Major**	NL	NL	NL
2-Nonanone; Methyl n-heptyl ketone	821-55-6	13187	x		**Major**	**Major**	**Major**	NL	NL	**Major**
Dimethyl sulfide; Methyl sulfide	75-18-3	1068			**Major**	**Major**	**Major**	NL	NL	NL
δ-Decalactone; 5-Decalactone	705-86-2	12810	x		**Major**	**Major**	?	NL	NL	NL
δ-Dodecalactone	713-95-1	12844	x	150	**Major**	Minor	NL	NL	NL	NL
Hexanedione: 2,3-Hexanedione 3,4-Hexanedione	3848-24-6 4437-51-8	19707 62539	x		**Major**	Minor	Minor	NL	NL	NL
1,1-Diethoxyethane; Acetal	105-57-7	7765			**Major**	Minor	Minor	NL	NL	NL
2-Heptanone; Methyl amyl ketone; MAK	110-43-0	8051	x	100	**Major**	Minor	Minor	NL	NL	NL
Isobutanal; Isobutyraldehyde	78-84-2	6561	x	100	**Major**	Minor	Minor	NL	NL	NL
Acetaldehyde*	75-07-0	177			**Major**	NL	NL	**Major**	Minor	NL
Ethyl lactate; Ethyl 2-hydroxypropionate	97-64-3	637513			**Major**	NL	NL	Minor	Minor	NL
Propanoic acid; Propionic acid	79-09-4	1032	x		**Major**	NL	NL	*Minor*	?	NL

Chemical Name	CASRN	PubChem CID	Dry [b] popcorn	Sweet [c] Butter at: (˚C)	Butter Flavor [d] Ridgway (Liq. or Paste)	QC Room Ridgway [e]	Mixing Room Ridgway [e]	Mixing Room Sioux City [f] (Liq. or Paste)	Mixing Room Sioux City (Powder)	Mixing Room Jasper [g]
Minor Constituents Identified in Butter Flavoring and Found in Room Air										
Dodecane	112-40-3	8182	x		Minor	**Major**	**Major**	Minor	Minor	NL
Tridecane	629-50-5	12388	x		Minor	Minor	**Major**	Minor	**Major**	NL
Silicone compounds (decamethylcyclopentasiloxane; polydimethylsiloxane; and Dow corning 345) [possible artifacts from analytical method or from anti-foam agents used with flavorings, e.g., polydimethylsiloxane)	541-02-6	10913			Minor	**Major**	**Major**	**Major**	**Major**	
Hexamethylcyclotrisiloxane*	541-05-9	10914			Minor	*Minor*	Minor	Minor	Minor	NL
Methyl propionic acid ester [esters of isobutyric acid? methyl propanoate? isoamyl, 2-methylbutyl, and phenethyl isobutyrates]*	554-12-1	11124			Minor	Minor	*Minor*	Minor	Minor	NL
Methanol*	67-56-1	887			Minor	**Major**	**Major**	NL	NL	NL
δ-Undecalactone	710-04-3	61204		150	Minor	***Major***	*Minor*	NL	NL	NL
Formaldehyde	50-00-0	712			Minor	?	?	NL	NL	NL
Pentanal; Valeraldehyde	110-62-3	8063	x	100	Minor	NL	NL	?	Minor	NL
Constituents Identified in Butter Flavoring but NOT Room Air										
1,1-Diethoxybutane; Butyraldehyde diethyl acetal	3658-95-5	77225			**Major**	NL	NL	NL	NL	NL
2-Methyl-1,3-dioxolane	497-26-7	10342			**Major**	NL	NL	NL	NL	NL
Hexanoic acid; Caproic acid	142-62-1	8892	x	100	**Major**	NL	NL	NL	NL	NL
Aliphatic esters/oxygen compounds (Some were identified separately in room air samples.)	N/A	N/A			**Major**	NL	NL	NL	NL	NL
Acetylpyridine: 2-Acetylpyridine 3-Acetylpyridine 4-Acetylpyridine	30440-88-1 350-03-8 1122-54-9	14286 9589 14282	x		**Major**	NL	NL	NL	NL	NL
Alkyldioxolanes	N/A	N/A			**Major**	NL	NL	NL	NL	NL
Butyl isobutyrate	97-87-0	7353			**Major**	NL	NL	NL	NL	NL
$C_{18}H_{14}O_4$ isomers: Dihydroxydimethylhexanedione?	N/A	N/A			**Major**	NL	NL	NL	NL	NL
Decanoic acid; Capric acid	334-48-5	2969		150	**Major**	NL	NL	NL	NL	NL
Ethyl decanoate; Ethyl caprate	110-38-3	8048	x		**Major**	NL	NL	NL	NL	NL
Ethyl hexanoate; Ethyl caproate	123-66-0	31265			**Major**	NL	NL	NL	NL	NL
Ethyl octanoate; Ethyl caprylate	106-32-1	7799			**Major**	NL	NL	NL	NL	NL

Chemical Name	CASRN	PubChem CID	Dry [b] popcorn	Sweet [c] Butter at: (˚C)	Butter Flavor [d] Ridgway (Liq. or Paste)	QC Room Ridgway [e]	Mixing Room Ridgway [e]	Mixing Room Sioux City [f] (Liq. or Paste)	Mixing Room Sioux City (Powder)	Mixing Room Jasper [g]
Ethyl propionate	105-37-3	7749			**Major**	NL	NL	NL	NL	NL
Ethyl vinyl ether; Vinamar	109-92-2	8023			**Major**	NL	NL	NL	NL	NL
1-Hydroxy-2-butanone; 2-Oxobutanol	5077-67-8	521300	**x**		**Major**	NL	NL	NL	NL	NL
Methyl hexanoate; Methyl caproate	106-70-7	7824			**Major**	NL	NL	NL	NL	NL
Methylpentenal (2-methyl-2-pentenal)	623-36-9	5319754			**Major**	NL	NL	NL	NL	NL
γ-Nonalactone; Coconut aldehyde	104-61-0	7710	**x**		**Major**	NL	NL	NL	NL	NL
γ-Decalactone	706-14-9	12813			Minor	NL	NL	NL	NL	NL
Dodecanoic acid; Lauric acid	143-07-7	3893	**x**	100	***Major***	NL	NL	NL	NL	NL
Ethyl dodecanoate; Ethyl laurate	106-33-2	7800			Minor	NL	NL	NL	NL	NL
Propanal; Propionaldehyde	123-38-6	527			***Major***	NL	NL	NL	NL	NL
Vanillin; 4-Hydroxy-3-methoxybenzaldehyde	121-33-5	1183	**x**		Minor	NL	NL	NL	NL	NL
Dimethyl sulfoxide; DMSO; Methyl sulfoxide	67-68-5	679			***Major***	NL	NL	NL	NL	NL
Isobornyl isovalerate; Isobornyl isopentanoate	7779-73-9	No CID			***Major***	NL	NL	NL	NL	NL
Maltol [also a soybean volatile]	N/A	N/A		200	***Major***	NL	NL	NL	NL	NL
Nitrogen compounds?	N/A	N/A			*Minor*	NL	NL	NL	NL	NL
n-Pentanoic acid; Valeric acid	109-52-4	7991	**x**	150	?	NL	NL	NL	NL	NL
n-Pentanol; Amyl alcohol	71-41-0	6276	**x**		?	NL	NL	NL	NL	NL
Xylene - o-Xylene - m-Xylene - p-Xylene [Peaks for butter flavoring are either not well separated from butyric acid or are <<0.05. See Ethylbenzene/Xylenes.]	95-47-6 108-38-3 106-42-3	7237 7929 16821	**x**		***Major***	NL	NL	NL	NL	NL
Chemicals Identified in Room Air but <u>NOT</u> Butter Flavoring										
Isopropyl alcohol; 2-Propanol; Isopropanol	67-63-0	3776			NL	**Major**	**Major**	**Major**	**Major**	NL
Propane	74-98-6	6334			NL	**Major**	**Major**	**Major**	**Major**	NL
Tetradecane [peaks not numbered for Ridgway chromatograms]	629-59-4	12389	**x**		NL	**Major**	**Major**	Minor	Minor	NL
Acetone; 2-Propanone	67-64-1	180			NL	**Major**	**Major**	?	Minor	NL
Furfural; 2-Furaldehyde; 2-Hydroxymethylfuran	98-01-1	7362	**x**	150	NL	Minor (w/ octane)	?	**Major**	**Major**	NL
Hexane	110-54-3	8058		100	NL	?	?	**Major**	**Major**	NL

Chemical Name	CASRN	PubChem CID	Dry [b] popcorn	Sweet [c] Butter at: (˚C)	Butter Flavor [d] Ridgway (Liq. or Paste)	QC Room Ridgway [e]	Mixing Room Ridgway [e]	Mixing Room Sioux City [f] (Liq. or Paste)	Mixing Room Sioux City (Powder)	Mixing Room Jasper [g]
2-Ethyl-2-methyloxirane; 1,2-Epoxy-2-methylbutane (2-R- epimer); (2-S- epimer) [+ ethyl acetate in Ridgway room air]	30095-63-7	7830021 7832547			NL	**Major**	**Major**	NL	NL	NL
Octamethylcyclotetrasiloxane	556-67-2	11169			NL	NL	NL	?	?	NL
Octanone: 2-Octanone 3-Octanone	111-13-7 106-68-3	8093 246728		150	NL	NL	NL	Minor	?	NL
Octanal	124-13-0	454	**x**	150	NL	Minor	*Minor*	**Major**	?	NL
β-Pinene	127-91-3	14896			NL	NL	NL	Minor	Minor	NL
Benzene [with Isopropyl acetate]	71-43-2	241			NL	NL	NL	**Major**	Minor	NL
Butane/Butene	106-97-8	7843			NL	**Major**	**Major**	NL	NL	NL
Butyl Cellosolve®; Ethylene glycol monobutyl ether	111-76-2	8133			NL	Minor	*Minor*	Minor	**Major**	NL
C_8 aliphatic hydrocarbons	N/A	N/A			NL	**Major**	Minor	Minor	Minor	NL
C_9-C_{16} mostly branched alkanes and aliphatic hydrocarbons plus some C_9-C_{10} alkylbenzenes	N/A	N/A			NL	***Major***	**Major**	Minor	Minor	NL
p-Dichlorobenzene	106-46-7	4685			NL	**Major**	Minor	?	?	NL
2,2-Dimethoxybutane; Butyraldehyde dimethyl acetal	3453-99-4	137941			NL	Minor	**Major**	NL	NL	NL
Isooctane;	26635-64-3	11594			NL	Minor	Minor	**Major**	Minor	NL
1-Methoxy-2-propanol; Propylene glycol monomethyl ether	107-98-2	7900			NL	**Major**	**Major**	**Major**	Minor	NL
3-Methyl-3-buten-2-one; isopropenyl methyl ketone	814-78-8	13143			NL	Minor	Minor	**Major**	*Minor*	NL
α-Methylstyrene (methylstyrene isomer)	98-83-9	7407			NL	**Major**	*Minor*	Minor	?	NL
Nonane	111-84-2	8141	**x**	100	NL	**Major**	**Major**	**Major**	Minor	NL
Aliphatic, oxygen compounds, (methoxybutene?); two compounds not have numerous NLM database records: 2-methylbutanal (96-17-3) and 3-methylbutanal (isovaleraldehyde, 590-86-0)	N/A	N/A			NL	Minor	**Major**	NL	NL	NL
Bromoethane; Ethyl bromide	74-96-4	6332			NL	**Major**	?	NL	NL	NL
3-Buten-2-one; Methyl vinyl ketone	78-94-4	6570			NL	Minor	**Major**	Minor	Minor	NL
C_5H_{10} isomers	N/A	N/A			NL	NL	NL	**Major**	Minor	NL
C_6 aliphatic hydrocarbons	N/A	N/A			NL	NL	NL	**Major**	**Major**	NL
N,N-Dimethylformamide	68-12-2	6228			NL	Minor	**Major**	NL	NL	NL

Chemical Name	CASRN	PubChem CID	Dry [b] popcorn	Sweet [c] Butter at: (˚C)	Butter Flavor [d] Ridgway (Liq. or Paste)	QC Room Ridgway [e]	Mixing Room Ridgway [e]	Mixing Room Sioux City [f] (Liq. or Paste)	Mixing Room Sioux City (Powder)	Mixing Room Jasper [g]
Dimethylstyrene; p, α-Dimethylstyrene	1195-32-0	62385			NL	NL	NL	Minor	**Major**	NL
Heptanal; Heptaldehyde	111-71-7	8130		100	NL	NL	NL	***Major***	Minor	NL
Isopentane	78-78-4	6556			NL	NL	NL	**Major**	**Major**	NL
Octane	111-65-9	356		100	NL	**Major**	Minor	NL	NL	NL
Heptane*	142-82-5	8900		100	NL	Minor	Minor	Minor	Minor	NL
Naphthalene	91-20-3	931	**x**		NL	Minor	Minor	Minor	Minor	NL
Propylene glycol methyl ether acetate; PGMEA	108-65-6	7946			NL	Minor	Minor	Minor	Minor	NL
1,1,1-Trichloroethane; Methyl chloroform	71-53-6	6278			NL	Minor	Minor	*Minor*	?	NL
2-Butenal; Crotonaldehyde [(E)-Crotonaldehyde (123-73-9) same PubChem]	4170-30-3	447466			NL	Minor	Minor	NL	NL	NL
Butyl acrylate	141-32-2	8846			NL	NL	NL	*Minor*	*Minor*	NL
tert-Butyl peroxide?; Di-tert-butyl peroxide	110-05-4	8033			NL	Minor	Minor	NL	NL	NL
Butyronitrile	109-74-0	8008			NL	NL	NL	Minor	*Minor*	NL
C_7 aliphatic hydrocarbons	N/A	N/A			NL	NL	NL	Minor	Minor	NL
Chloromethane; Methyl chloride	74-87-3	6327			NL	?	?	NL	NL	NL
Formic acid	64-18-6	284			NL	NL	NL	*Minor*	Minor	NL
Nitromethane ***Carbon disulfide*** 1,1,2-Trichloro-1,2,2-trifluoroethane (CFC 113)	75-52-5 75-15-0 76-13-1	6375 6348 6428			NL	Minor	Minor	NL	NL	NL
2-Propoxyethanol; Ethylene glycol monopropyl ether	2807-30-9	17756			NL	NL	NL	Minor	Minor	NL
Trichlorofluoromethane; CFC-11	75-69-4	6389			NL	NL	NL	?	Minor	NL
Chemicals Identified in Room Air and Questionable in Butter Flavoring										
Perchloroethylene; 1,1,2,2-Tetrachloroethene	127-18-4	31373			***Major***	NL	NL	**Major**	**Major**	NL
Methylene chloride; Dichloromethane	75-09-2	6344			***Major***	NL	NL	*Minor*	*Minor*	NL
Pentane* [not well separated from diacetyl peak in butter flavor]	109-66-0	8003		100	***Major***	**Major**	Minor	**Major**	**Major**	NL
Hexanal* [also a soy volatile]	66-25-1	6184	**x**	100	*Major*	Minor	Minor	Minor	**Major**	NL
Undecane	1120-21-4	14257		100	*Minor*	Minor	**Major**	**Major**	**Major**	NL
Toluene	108-88-3	1140	**x**		*Minor*	**Major**	**Major**	**Major**	**Major**	NL
Ethylbenzene	100-41-4	7500			*Minor*	**Major**	Minor	**Major**	**Major**	NL
Nonanal	124-19-6	31289	**x**	100	*Minor*	Minor	Minor	**Major**	**Major**	NL

Chemical Name	CASRN	PubChem CID	Dry [b] popcorn	Sweet [c] Butter at: (˚C)	Butter Flavor [d] Ridgway (Liq. or Paste)	QC Room Ridgway [e]	Mixing Room			
							Ridgway [e]	Sioux City [f] (Liq. or Paste)	Sioux City (Powder)	Jasper [g]
α-Pinene [peaks not numbered for Ridgway chromatograms]	80-56-8	6654			*Minor*	?	Minor	**Major**	**Major**	NL
Diethyl phthalate*	84-66-2	6781			*Minor*	**Major**	Minor	*Minor*	*Minor*	NL
Dimethyl phthalate*	131-11-3	8554			*Minor*	**Major**	Minor	NL	NL	NL
C_7-C_{10} aliphatic aldehydes [Straight-chain aldehydes, octanal, nonanal, and decanal are listed separately.]	N/A	N/A			*Minor*	Minor	*Minor*	NL	NL	NL
Decanal	112-31-2	8175	**x**	100	*Minor*	NL	NL	Minor	Minor	NL

*Sometimes present in media and/or system blanks.

**May be present as a thermal decomposition product and/or impurity of ethanol.

[a] Relative level of VOCs estimated from thermal desorption chromatograms based on peak height as reported by Kanwal et al. (2004) [Sioux City, IA, HHE], Sahakian et al. (2003) [Ridgway, IL, HHE], and Gomaa et al. (2001) [Jasper, MO, HEE] {included in Kanwal et al. (2006) [HHE, p. 92]}; ***Bold Italics*** (column 1) = Some respiratory effects data available and summarized in Table II below; **Major** = major constituent based on relative peak height; Minor = minor constituent based on relative peak height; ***Major*** = possible major constituent (not labeled on chromatogram) based on peak location compared to analyte list; *Minor* = possible minor constituent (not labeled on chromatogram) based on peak location compared to analyte list; ? = shown on analyte list, but no peak was visible on chromatogram; NL = not labeled on chromatogram or on analyte list; and N/A = not applicable.

[b] Identified in VOCs from microwave popped popcorn without oil or butter flavoring (Buttery et al., 1997; Rengarajan and Seitz, 2004)

[c] Fresh sweet butter (as opposed to rancid butter which has a higher concentration of butyric acid); temperature (Celsius) to which butter was heated (5 hours) to produce VOCs (Lee et al., 1991).

[d] Ridgway facility: Bulk butter flavoring (liquid or paste) was heated to 50 ˚C and headspace samples analyzed (Nov. 2002); the range of peak heights on the chromatogram was <0.5 to 9.5 x 10^6; the cut-off between major and minor peaks was set at 3 x 10^6 (visible point of separation). Some peak numbers on chromatograms were slightly out of order and occasionally more unlabeled peaks were seen between those that were labeled than expected based on the corresponding analyte list (e.g. four peaks between peak 70 and 74).

[e] Room air, the range of peak heights was <0.1 to 1.9 x 10^6. The cut-off between major and minor peaks was 0.3 x 10^6 (visible point of separation).

[f] Sioux City facility: Mixing room air, the range of peak heights was <0.05 x 10^6 to >0.95 x 10^6. The cut-off between major and minor was 0.15 x 10^6 (visible point of separation).

[g] Jasper facility: Room air from all areas of the facility unless otherwise noted; the range of peak heights was < 0.2 to >3 x 10^6. The chemical identities for only a few peaks were shown on the chromatogram (no list of analytes available), and all of them were considered major.

[h] Levels of diacetyl were much lower when butter was heated to ≥150 ˚C (appears to decompose). Thermal decomposition of diacetyl gave methane, carbon monoxide, ketene (CH_2=C=O), and ketene polymers, among other products, possibly via a free-radical mechanism (Rice and Walters, 1939). Ketene is as acutely toxic to the lungs as phosgene (Wooster et al., 1946).

Table II. Brief respiratory effects data for compounds identified in butter flavoring volatiles and/or room air of popcorn plants

Compound (CAS & PubChem No.)	Potential for Nasal and Lung Toxicity
Acetaldehyde (75-07-0; CID: 177)	**Upper respiratory tract:** at the high concentration, inhalation exposure increased nasal tumors in rats and laryngeal and nasal tumors in hamsters. 52-week exposure at concentrations up to 1500-3000 ppm caused labored breathing, degeneration of olfactory epithelium, hyper- and metaplastic changes in respiratory epithelium, and rhinitis in rats. Mucous membrane irritation and ciliastatic effects may occur in the upper respiratory tract of humans at >100-200 ppm (HSDB #230, 2005). **Lungs:** Lung tumors were not induced by intratracheal dosing of hamsters, but "peribronchiolar ademomatoid lesions" were observed in the lung. Ciliotoxic and mucus coagulating effects were seen. At the end of a 5-week study, rats inhaling 243 ppm showed increased functional residual capacity, residual volume, total lung capacity, and respiratory frequency. Damage to the peripheral regions of the lung parenchyma affected small airways or altered pulmonary elastic properties (HSDB #230, 2005). Acetaldehyde may cause oxidative DNA damage in rat lung tissues (Xi et al., 2004). It may induce bronchoconstriction in human asthmatics when inhaled (Sanchez-Toril et al., 2000).
Acetic acid (64-19-7; CID: 176)	Several persons acutely exposed to high concentrations after a glacial (100%) acetic acid spill developed reactive airways dysfunction syndrome (RADS) (Kern, 1991). High acute exposure in rats failed to induce RADS (Ariel et al., 1998).
Acetonitrile* (75-05-8; CID: 6342)	Vapors are irritating to eyes, nose, and throat. Massive exposures induce respiratory asphyxiation due to cyanide poisoning. Rats inhaling the LC_{50} or LC_{84} developed pulmonary edema (HSDB #42, 2005). In a 2-year study in rats and mice, equivocal evidence of hepatic tumors but no lung toxicity was reported. In a 13-week study no clear histopathological effects were observed, but some rats died early with pulmonary congestion and edema and hemorrhage in the lung and brain (NTP TR-447, 1996).
Bromoethane; Ethyl bromide (74-96-4; CID: 6332)	Respiratory irritant. Only highest dose (400 ppm) in 2-year carcinogenesis bioassay induced nasal and alveolar epithelial hyperplasia (HSDB, #532, 2005).
2-Butenal; Crotonaldehyde (4170-30-3; CID: 447466) [(*E*)-Crotonaldehyde (123-73-9)]	A strong respiratory irritant, causing pulmonary edema at high concentrations (HSDB #252, 2005).
3-Buten-2-one; Methyl vinyl ketone (78-94-4; CID: 6570)	Twelve inhalation exposures over 12 days caused upper airway irritation and necrosis in mice and rats; rats were more sensitive (Morgan et al. [NTP], 2000).
Butyl acrylate (141-32-2; CID: 8846)	Acrylate esters (hydrolyzed in nasal and lung tissues to acrylic acid) and acrylic acid itself induced olfactory epithelial lesions in rodents; respiratory epithelium was "relatively unaffected." Esters induced glutathione (GSH) and nonprotein GSH depletion (Miller et al., 1981a, 1981b; Stott and McKenna, 1985; Vodicka et al., 1990).
Butyl Cellosolve®; Ethylene glycol monobutyl ether; EGBE; 2-Butoxyethanol (111-76-2; CID: 8133)	Pulmonary edema was reported in acute oral poisoning of an alcoholic man (Bauer et al., 1992). In a 2-year inhalation study in mice and rats, hyaline degeneration of the olfactory epithelium was induced; there were no effects on the lungs (NTP TR-484, 2000).
tert-Butyl peroxide?; Di-*tert*-butyl peroxide (110-05-4; CID: 8033)	Low acute inhalation as well as dermal and eye toxicity (BIBRA, 1990)
Butyric acid (107-92-6; CID: 264)	Butyric acid and Na butyrate were included in a structure-activity model of compounds with documented ability to induce respiratory sensitization (Graham et al., 1997). Butyric acid, octanoic acid, and other small fatty acids induced maternal respiratory toxicity in rats gavaged on gestation days 6-15 (Narotsky et al., 1991, 1994). Severe lung changes were observed in rabbits after inhalation of aerosol (40 mg/L = 40,000 mg/m^3) for 1.5 hours (Danishevskii and Monastyrskaya, 1960). Butyric acid may have a pathophysiological role in some tumors and obstructive lung diseases or upper respiratory tract cancers. In *in vitro* studies with cell lines from human nasopharyngeal carcinomas, Na butyrate upregulated expression of IL-6 and IL-2R□. Elevated serum IL-6 has been associated with several human cancers, including lung cancer, and chronic lung diseases, including bronchiolitis obliterans after lung transplantation (Chow et al., 2003; Lu et al., 2002; Wang et al., 1999).
Butyronitrile (109-74-0; CID: 8008)	REL=22 mg/m^3. Symptoms of human exposure to nitriles include bronchial tightness and respiratory distress (NIOSH, 1978).

Compound (CAS & PubChem No.)	Potential for Nasal and Lung Toxicity
Chloromethane; Methyl chloride (74-87-3; CID: 6327)	May be corrosive at high concentrations producing pulmonary edema with lesions and disturbing surfactant metabolism (Huguenard et al., 1975). The mortality incidence of 24 persons accidentally exposed to methyl chloride 32 years previously, showed increased risk ratios for cardiovascular disease and all cancers, including lung (Rafnsson and Gudmundsson, 1997). Induced more severe and extensive necrosis of the olfactory epithelium in rats than in mice in a 6-week inhalation study and damaged several organs (Eustis et al. [NTP], 1988).
Decanal (112-31-2; CID: 8175)	Low-molecular-weight aldehydes are irritating to the membranes of the nasal and oral passages and the upper respiratory tract, inducing bronchial constriction, choking, and coughing (HSDB #288, 2002). Longer-chain aldehydes and carboxylic acids might be expected to be less irritating than the lower-chain members based on observations that nasal pungency (irritation) declined in the homologous series of aldehydes (butanal through octanal) and carboxylic acids (formic, butyric, hexanoic, and octanoic) in volunteer anosmics (Cometto-Muniz et al., 1998).
p-Dichlorobenzene (106-46-7; CID: 4685)	One of 11 common VOCs found in human blood (U.S. population in the third NHANES study in 1988-1994) that was still associated with reduced pulmonary function after adjustment for smoking (Elliott et al., 2006). Also one of the most common VOCs found in indoor air and in exhaled human breath (e.g., Wallace et al., 1991).
N,N-Dimethylformamide (68-12-2; CID: 6228)	No involvement of the respiratory tract in the toxicity (HSDB #78, 2005).
Dimethyl sulfide; Methyl sulfide (75-18-3; CID: 1068)	No reports of lung toxicity were found; however, a worker exposed to high concentrations in a confined space died of hypoxia (Terazawa et al., 1991). Vapor is moderate eye, nose, and throat irritant (HSDB #356, 2002). The 24-hour LC_{50} in rats (4 hour exposure) was 40,250 ppm (Tansy et al., 1981).
Ethylbenzene (100-41-4; CID: 7500)/Xylenes *o*-Xylene (95-47-6; CID: 7237). *m*-Xylene (108-38-3; CID: 7929) *p*-Xylene (106-42-3; CID: 16821)	In a 2-year bioassay in rats and mice, 750 ppm induced alveolar epithelium metaplasia, alveolar/bronchiolar adenoma, and liver toxicity in male mice and liver tumors in female mice (Chan et al. [NTP], 1998; NTP TR-466, 1999).
Ethyl vinyl ether; Vinamar (109-92-2; CID: 8023)	Probably not a respiratory tract irritant; 4-hour LC_{50} in rats was >21,200 mg/m^3; 15-minute LC_{50} in mice was 324,000 mg/m^3 (ChemIDplus, 2004)
Formaldehyde (50-00-0; CID: 712)	Induced nasal squamous cell carcinomas in rats and mice but not lung lesions in a 2-year cancer bioassay (Kerns et al. [CIIT], 1983). Strength of evidence for nasopharyngeal/sino-nasal cancer = strong, pulmonary edema = good, allergic asthma = good, and laryngeal and lung cancer = limited (CHE, undated).
Formic acid (64-18-6; CID: 284)	A 13-week inhalation study in rats and mice reported squamous metaplasia and degeneration in olfactory and respiratory epithelia (NTP TOX-19, 1992).
Furfural; 2-Furaldehyde; 2-Hydroxymethylfuran (98-01-1; CID: 7362)	Human respiratory irritant. 13 week inhalation exposure of guinea pigs and 4 week exposure of rats induced nasal histopathology. Induced pulmonary irritation, parenchymal injury, and regenerative proliferation of type II pneumocytes in a 30-day rat inhalation study (HSDB #542, 2006).
Hexanal* (66-25-1; CID: 6184)	Inhalation of 2 or 10 ppm did not affect pulmonary function but did cause eye and nose discomfort and headaches in humans (Ernstgard et al., 2006).
Hexanoic acid; Caproic acid (142-62-1; CID: 8892)	Mild to strong skin, eye, and respiratory tract irritant. No inhalation studies were available (BUA, 2005; Canadian Centre for Occupational Health and Safety, 1990; HSDB #6813, 2006)

Compound (CAS & PubChem No.)	Potential for Nasal and Lung Toxicity
Hydrogen sulfide (7783-06-4; CID: 402) [not listed in Table I; requires use of a specific analytical method to detect]	Mentioned in the literature as having induced bronchiolitis obliterans (e.g., Boswell and McCunney, 1995), but only one original published report was found. Two patients had RADS and one had chemical pneumonitis with bronchiolitis obliterans and fibrosis. The latter case did not improve with treatment (Malbrain et al., 1997). Four years after poisoning by inhalation, a patient who had suffered pulmonary edema developed pulmonary fibrosis (Duong et al., 2001). Several community-based epidemiological studies (one in South Sioux City, NB) noted increased incidences of respiratory diseases in communities with compared to those without H_2S emission sources (e.g., Bates et al., 1998; Campagna et al., 2004; Durand and Wilson, 2006; Legator et al., 2001). High concentrations induced nasal lesions in rodents and anosmia and dysosmia in humans (Brenneman et al. [CIIT], 2002). Concentrations up to 80 ppm for ≥90 days caused nasal (olfactory neuronal loss) and lung toxicity in rats and mice. Rats developed bronchiolar epithelial hypertrophy and hyperplasia (Dorman et al. [CIIT], 2004). **Note:** Levels of hydrogen sulfide and □-butyrolactone emitted from popping unflavored popcorn were 1000-fold higher than levels of most other compounds emitted. Special methods were needed to trap and quantify H_2S (Buttery et al., 1997).
Isobutanal; Isobutyraldehyde (78-84-2; CID: 6561)	Eye and respiratory tract irritant. Inhalation of high doses induced fatal pulmonary edema in animals (HSDB #614, 2003). Exposure to ≥1,000 ppm for up to 105 weeks induced severe nonneoplastic nasal lesions, abnormal respiratory sounds, and/or slowed respiration without lung lesions in rats and mice (Abdo et al., 1998; NTP TR-472, 1999).
Ketene; Ethenone (463-51-4; CID: 10038) [not listed in Table I but it is a thermal decomposition product of diacetyl]	Acute toxicity values in several species may be found in the IDLH documentation. Death was from pulmonary edema (NIOSH, 1996). Acute inhalation toxicity is comparable to that of phosgene and hydrogen cyanide (Wooster et al., 1946). Repeated exposures may lead to tolerance; delayed toxicity resembles that of phosgene-emphysema and fibrosis (HSDB #633, 2005). **Note:** Ketene (CH_2=C=O) and ketene polymers are among the thermal decomposition products of diacetyl (Rice and Walters, 1939); appears that diacetyl in sweet butter decomposes when heated above 150 ˚C; diacetyl levels are lower than those at 100 ˚C (Lee et al., 1991). A NIOSH or OSHA colorimetric method recommended for ketene analysis was not used in the health hazard evaluations of the microwave popcorn plants (i.e., OSHA CSI sampling method [SKC Inc., 2007] or NIOSH 2/S92 [OSHA, 1992; Sigma-Aldrich Co., 1999).
Limonene *d,l*-Limonene; Dipentene (138-86-3; CID: 22311) (*R*)-(+)-Limonene; *d*-Limonene (5989-27-5; CID: 440917) (*S*)-(-)-Limonene; *l*-Limonene (5989-54-8; CID: 439250)	Exposure to 450 mg/m^3 (~81 ppm) for 2 hours caused decreased vital capacity in exercising human volunteers (Falk-Filipsson et al, 1993). The sensory irritation threshold is >80 ppm for humans and >100 ppm for mice. Mice showed mild broncho-constriction after short exposures to either enantiomer at >1,000 ppm (Larsen et al., 2000). *d*-Limonene is readily oxidized by ozone and its oxidation products cause significant upper airway irritation at concentrations well below NOELs of the unoxidized form (Wolkoff et al., 2000). Mice exposed to ozone-limonene reaction products (original limonene concentration 51 ppm [~280 mg/m^3]) showed both upper airway irritation and "airflow limitation that persisted for at least 45 min post exposure" (Rohr et al., 2002).
1-Methoxy-2-propanol; Propylene glycol monomethyl ether; PGME (107-98-2; CID: 7900)	No lung toxicity was observed in rats or mice in a 2-year cancer bioassay (Spencer et al., 2002) or in rats inhaling vapors in a 2-generation reproductive study (Carney et al., 1999).
3-Methyl-3-buten-2-one; Isopropenyl methyl ketone (814-78-8; CID: 13143)	Eyes, nose and throat irritant; rats, guinea pigs, and rabbits given 20 or 100 seven-hour inhalation exposures to 30 ppm or 15 ppm respectively, had ocular and nasal irritation and kidney damage (HSDB #1164, 2002).
Methylene chloride; Dichloromethane (75-09-2; CID: 6344)	Inhalation produced alveolar/bronchiolar neoplasms and hepatocellular neoplasms in mice. Rat tumors did not involve the lungs [NTP bioassay] (HSDB #66, 2005).
Methylstyrene isomer α-Methylstyrene (98-83-9; CID: 7407)	Toxicity in rodents inhaling high concentrations for 9-12 days did not involve the lungs (Morgan et al. [NTP], 1999).
Naphthalene (91-20-3; CID: 931)	Rat inhalation induced nasal and lung tumors [NTP bioassay] (Abdo et al., 2001).
Nitromethane (75-52-5)/Carbon disulfide/1,1,2-Trichloro-1,2,2-trifluoroethane (CFC 113)	Nitromethane is an eye and respiratory irritant; it is neurotoxic and carcinogenic (HSDB #106, 2006). Carbon disulfide's toxicity does not involve the respiratory tract (HSDB #52, 2005).

Compound (CAS & PubChem No.)	Potential for Nasal and Lung Toxicity
Octanal (124-13-0; CID: 454)	Low-molecular-weight aldehydes are irritating to the membranes of the nasal and oral passages and the upper respiratory tract, inducing bronchial constriction, choking and coughing (HSDB #5147, 2003).
Perchloroethylene; 1,1,2,2-Tetrachloroethene (127-18-4; CID: 31373)	Occupational asthma reported in humans acutely exposed to high concentrations (Boulet, 1988).
Propanal; Propionaldehyde (123-38-6; CID: 527)	Mice, guinea pigs, and rabbits that inhaled high concentrations developed fatal pulmonary edema (HSDB #1193, 2003).
Propanoic acid; Propionic acid (79-09-4; CID: 1032)	No inhalation toxicity studies were found, but one case of mild cough and asthmatic response was reported; corrosive, caused severe eye and skin burns, and was irritating to the eyes, skin, and lungs (HSDB #1192, 2006).
Propylene glycol methyl ether acetate; PGMEA (108-65-6; CID: 7946)	Short-term inhalation study (300-3000 ppm) in rats showed no lung toxicity (PGMEA is rapidly hydrolyzed to PGME and acetic acid) (Miller et al., 1984).
2-Propoxyethanol; Ethylene glycol monopropyl ether (2807-30-9; CID: 17756)	Rabbits tolerated 2400 ppm 1-3 hours exhibiting only irritation of the mucous membranes. Rats inhaling an atmosphere saturated with ethylene glycol monopropyl ether for 7 hours showed lung, liver, and kidney injuries. Twelve 8-hour inhalation exposures to 600 ppm had no effect on mice and guinea pigs but were lethal to cats and rabbits. No lung toxicity in pregnant rats that inhaled 100-400 ppm for 6 hours on gestation days 6-15 (HSDB #6499, 2002).
Tetradecane (629-59-4; CID: 12389)	Lung injury appeared to be mediated by disruption of airway barrier epithelial function (Robledo et al., 2000) and inflammatory mechanisms (Harris et al., 1997; Wang et al., 2001). Inhalation of jet propulsion fuel 8 vapors (contains *n*-tetradecane) for 7 or 28 days increased pulmonary resistance in rats but no pathological evidence of lung injury (Pfaff et al., 1995).
1,1,1-Trichloroethane; Methyl chloroform (71-55-6; CID: 6278)	Inhalation for up to 90 days produced almost no toxicity in several species (HSDB #157, 2005).
Undecane (1120-21-4; CID: 14257)	Rats inhaling each of the alkanes *n*-nonane to *n*-tridecane showed cerebellar and liver toxicity but not lung toxicity (Nilsen et al., 1988).

*Sometimes present in media and/or system blanks.
NHANES = National Health and Nutrition Examination Survey

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