

**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**

*Prepared by:*  
Integrated Laboratory Systems, Inc.  
Research Triangle Park, NC  
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, [ $^{14}\text{C}$ ]-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 from 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 from 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 [ $^{14}\text{C}$ ]-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,  $^{12}\text{C}$ -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- $^{14}\text{C}$ ]-acetoin was detected as a mixture of 2,3-butanediol stereoisomers.

### Acute Toxicity

The  $\text{LC}_{50}$  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  $\text{LD}_{50}$  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<sup>3</sup>]) for six hours also produced significant necrosis of nasal epithelium at  $\geq 198$  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  $\alpha$ -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](http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/431-03-8.pdf)).

## Table of Contents

Abstract.....	i
Executive Summary .....	ii
1.0 Basis for Nomination .....	1
2.0 Introduction.....	1
2.1 Chemical Identification and Analysis .....	2
2.2 Physical-Chemical Properties.....	4
2.3 Commercial Availability.....	4
3.0 Production Processes.....	5
4.0 Production and Import Volumes .....	5
5.0 Uses .....	5
6.0 Environmental Occurrence and Persistence .....	6
7.0 Human Exposure.....	6
8.0 Regulatory Status .....	8
9.0 Toxicological Data .....	9
9.1 General Toxicology .....	9
9.1.1 Human Data .....	9
9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics.....	11
9.1.3 Acute Exposure.....	12
9.1.4 Short-term and Subchronic Exposure.....	16
9.1.5 Chronic Exposure.....	16
9.1.6 Synergistic/Antagonistic Effects .....	16
9.1.7 Cytotoxicity.....	17
9.2 Reproductive and Teratological Effects.....	17
9.3 Carcinogenicity .....	17
9.4 Initiation/Promotion Studies .....	17
9.5 Anticarcinogenicity .....	17
9.6 Genotoxicity .....	17
9.7 Cogenotoxicity.....	18
9.8 Antigenotoxicity .....	18
9.9 Immunotoxicity .....	18
9.10 Other Data.....	18
10.0 Structure-Activity Relationships .....	18
11.0 Online Databases and Secondary References.....	19
11.1 Online Databases .....	19
11.2 Secondary References .....	20
12.0 References.....	20
13.0 References Considered But Not Cited .....	25
Acknowledgements .....	26
Appendix A: Units and Abbreviations .....	27
Appendix B: Description of Search Strategy and Results .....	29
Appendix C. Volatile Organic Compounds (VOCs) in Popcorn Manufacturing.....	30

<b>Tables:</b>	
<b>Table 1</b>	<b>Concentrations of VOCs in Microwave Popcorn Manufacturing Plants.....8</b>
<b>Table 2</b>	<b>Acute Toxicity Values for Some Artificial Butter Flavoring Components..... 12</b>
<b>Table 3</b>	<b>Acute Inhalation Exposure to Artificial Butter Flavoring and Diacetyl ..... 14</b>



## 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](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](#)).

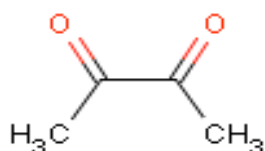
### Public Health Concern

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](#)

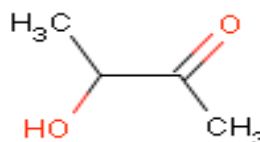
al., 2004; Kanwal, 2003 lett. [HETA 2002-0089]; Kanwal et al., 2004 [HETA 2001-0474-2943], 2006a; Kanwal and Martin, 2003 lett. [HETA 2001-0517]; Parmet and Von Essen, 2002 lett.).

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<sub>4</sub>H<sub>6</sub>O<sub>2</sub>; 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<sub>4</sub>H<sub>8</sub>O<sub>2</sub>; 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  
 $\gamma$ -Hydroxy- $\beta$ -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  $\delta$ -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  $\mu$ g/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)
<b><i>Diacetyl</i></b>		
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)*
<b><i>Acetoin</i></b>		
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 <sup>3</sup> @ 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](http://best-price.com), 2007); [QualityExtracts.com](http://QualityExtracts.com) (undated); [Alibaba.com](http://Alibaba.com) (2007); [Sysco.com](http://Sysco.com) (2006); [Country Kitchen SweetArt, Inc](http://CountryKitchenSweetArt.com) (undated); and [International Flavors and Fragrances, Inc.](http://InternationalFlavorsandFragrances.com) (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](http://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. Acetoin 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. Acetoin 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 µg/m<sup>3</sup> and 54 µg/m<sup>3</sup> 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 <sup>1</sup> ppm	Acetoin ppm	Acetaldehyde ppm	Acetic Acid ppm	Respirable Dust mg/m <sup>3</sup>
<b>Mixing Area</b>						
Ridgway, IL	Nov-2002	0.6	0.29	<0.02 <sup>2</sup>	0.19	0.48
Sioux City, IA	July-2002	0.08	<0.03	<0.02	<0.02	0.23 <sup>3</sup>
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 <sup>4</sup>	<0.6 <sup>4</sup>	0.19
<b>Processing and/or Packaging</b>						
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 <sup>3</sup>
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 <sup>4</sup>	<0.6 <sup>4</sup>	0.03
<b>QC Room</b>						
Ridgway, IL	Nov-2002	0.19	0.15	0.19	0.22	0.62
Sioux City, IA	July-2002	<DL <sup>5</sup>				0.03 <sup>3</sup>
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 <sup>4</sup>	<0.6 <sup>4</sup>	

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]

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

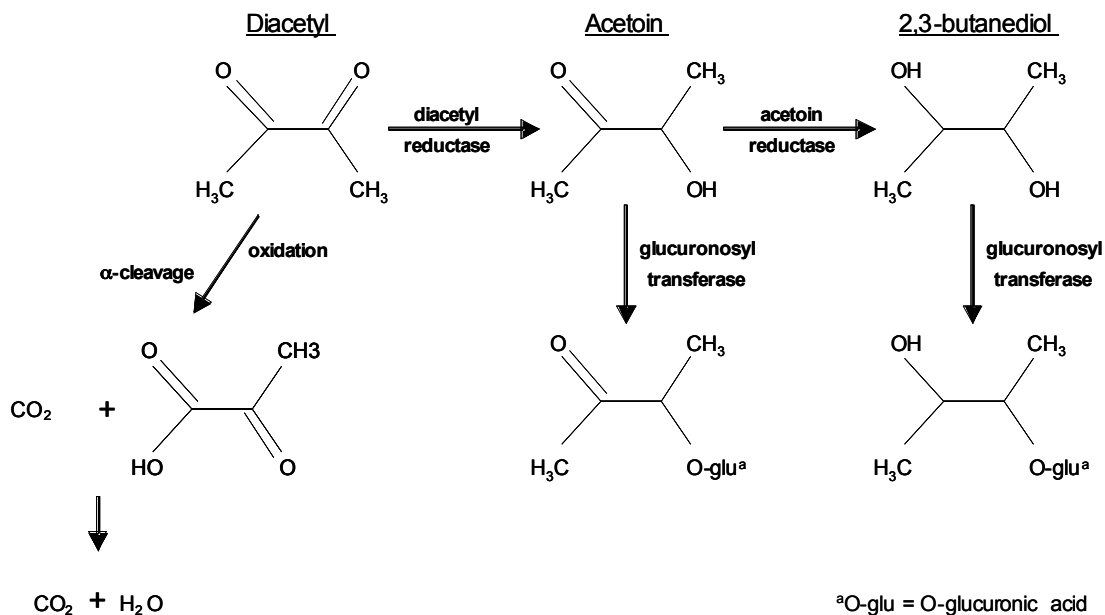
In a more recent study, risk for bronchiolitis obliterans was assessed at six microwave popcorn plants, using the Missouri facility as an index plant. An eight-hour time-weighted average concentration of diacetyl was used as an indicator of exposure to butter-flavoring VOCs. Mixers (those having mixed 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 ( $9 \times 10^{-4}$  mg) diacetyl to 3.7 nmol ( $3 \times 10^{-4}$  mg) acetoin and 6.3 nmol ( $6 \times 10^{-4}$  mg) butane-2,3-diol (Otsuka et al., 1996).

When administered to male Fischer 344 rats via intragastric gavage, a single dose of radiolabeled [ $^{14}\text{C}$ ]-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-<sup>14</sup>C]-acetoin was detected as a mixture of 2,3-butanediol stereoisomers. When acetoin (doses not provided) was i.p. injected into albino rats, <sup>12</sup>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 <sub>50</sub> /LC <sub>50</sub>	Reference(s)
<b>Diacetyl [431-03-8]</b>			
inh	rat (M, F; Wistar)	2.25 < LC <sub>50</sub> < 5.2 mg/L [4-hr] (2250 < LC <sub>50</sub> < 5200 mg/m <sup>3</sup> ; 639 < LC <sub>50</sub> < 1477 ppm)	BASF (1993)
i.p.	mouse (sex and strain n.p.)	LD <sub>50</sub> = 249 mg/kg (2.89 mmol/kg)	NTP (1994)
	rat (sex and strain n.p.)	LD <sub>50</sub> = 400-650 mg/kg (4.65-7.55 mol/kg)	ChemIDplus (2004a); NTP (1994)
oral	mouse (sex and strain n.p.)	LD <sub>50</sub> = 250 mg/kg (2.90 mmol/kg)	NTP (1994)
	rat (sex and strain n.p.)	LD <sub>50</sub> = 1580 mg/kg (18.35 mmol/kg)	ChemIDplus (2004a); NTP (1994)
	guinea pig (sex and strain n.p.)	LD <sub>50</sub> = 990 mg/kg (11.50 mmol/kg)	ChemIDplus (2004a); NTP (1994)
dermal	rabbit (sex and strain n.p.)	LD <sub>50</sub> > 5000 mg/kg (58.08 mmol/kg)	ChemIDplus (2004a); NTP (1994)
<b>Acetoin [513-86-0]</b>			
oral	rat (sex and strain n.p.)	LD <sub>50</sub> > 5000 mg/kg (56.75 mmol/kg)	ChemIDplus (2004b)
dermal	rabbit (sex and strain n.p.)	LD <sub>50</sub> > 5000 mg/kg (56.75 mmol/kg)	

Abbreviations: F = female(s); hr = hour(s); inh = inhalation; i.p. = intraperitoneal; LC<sub>50</sub> = concentration lethal to 50% of test animals; LD<sub>50</sub> = 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<sup>3</sup>]) for six hours also produced significant necrosis of nasal epithelium at  $\geq 198$  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
<i>Artificial Butter Flavoring</i>				
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	<p>Major peaks of vapors (headspace): diacetyl, acetic acid, butyric acid, acetoin, acetoin dimers, 2-nonanone, and <math>\delta</math>-alkyl lactones</p> <p>At the mid-constant and high-pulsed doses, one rat each died after exposure.</p> <p><u>Pulmonary findings:</u> 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.</p> <p><u>Nasal findings:</u> 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.</p>	<a href="#">Hubbs et al. (2002)</a>

**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
<i>Diacetyl</i>				
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	<p>Significant effects in the lung were not observed.</p> <p>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.</p> <p>Tracheal changes included the following:  <u>SEM</u>: multifocal denuding of basement membrane with cell swelling, loss of microvilli, and loss of ciliated cells in epithelium  <u>TEM</u>: epithelial necrosis, denuded basement membrane, and elongation of epithelial cells near foci of exposed basement membrane</p>	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	<p>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.</p> <p>At the mid and high doses, all rats died.</p> <p><u>Necropsy findings</u>: 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</p> <p><u>Microscopic findings</u>: 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</p> <p>2.25 &lt; LC<sub>50</sub> &lt; 5.2 mg/L</p>	<a href="#">BASF (1993)</a>

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 17 $\beta$ -dehydrogenase in human placenta under ultraviolet light (NTP, 1994). Diacetyl reduced the internalization of the lysosomal enzyme  $\alpha$ -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](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 <sup>*</sup>
BIOSIS Previews <sup>®</sup> (1969-present)	BIOSIS <sup>*</sup>
CAPLUS (1907-present)	CAPLUS
International Labour Office	CIS <sup>*</sup>
Toxicology Research Projects	CRISP <sup>*</sup>
Development and Reproductive Toxicology	DART <sup>®*</sup>
Environmental Mutagen Information Center File	EMIC <sup>*</sup>
Epidemiology Information System	EPIDEM <sup>*</sup>
Environmental Teratology Information Center File	ETIC <sup>*</sup>
Federal Research in Progress	FEDRIP <sup>*</sup>
Health Aspects of Pesticides Abstract Bulletin	HAPAB
Hazardous Materials Technical Center	HMTC <sup>*</sup>
International Pharmaceutical Abstracts (1970-present)	IPA <sup>*</sup>
MEDLINE (1951-present)	MEDLINE
Pesticides Abstracts	PESTAB <sup>*</sup>
Poisonous Plants Bibliography	PPBIB <sup>*</sup>
Swedish National Chemicals Inspectorate	RISKLINE
Toxic Substances Control Act Test Submissions	TSCATS <sup>*</sup>

\*These are also in TOXLINE. Missing are TOXBIB, NIOSHTIC<sup>®</sup>, NTIS.

#### National Archives and Records Administration

Code of Federal Regulations (CFR)

#### In-House Databases

Current Contents on Diskette<sup>®</sup>

The Merck Index, 2006, on CD-ROM

## 11.2 Secondary References

O'Neil, M.J., Ed. 2006. The Merck Index, 14<sup>th</sup> ed. Merck and Company, Inc., Whitehouse Station, NJ, pp. 12 and 504.

## 12.0 References

Akpınar-Elci, M., Travis, W.D., Lynch, D.A., and Kreiss, K. 2004. Bronchiolitis obliterans syndrome in popcorn production plant workers. *Eur Respir J*, 24:298-302.

Alibaba.com. 2007. Food flavors (Zhengzhou United Asia Trading Co., Ltd., China (Mainland)). Internet address:

[http://unitedasia.en.alibaba.com/offerdetail/52009934/Sell\\_Food\\_Flavour\\_Flavor\\_Flavouring\\_Flavoring\\_Essence.html](http://unitedasia.en.alibaba.com/offerdetail/52009934/Sell_Food_Flavour_Flavor_Flavouring_Flavoring_Essence.html). Last accessed on January 17, 2007.

Annemüller, G. 1973. Formation and decomposition of fermentation by-products in modern processes of discontinuous fermentation and maturing. *Lebensmittelindustrie*, 20(10):457-476. Abstract from CAPLUS 1974:58335.

BASF. 1993. Study on the acute inhalation toxicity LC<sub>50</sub> of Diacetyl FCC as a vapor in rats 4-hour exposure. Project No. 1310247/927010. BASF Aktiengesellschaft, Department of Toxicology, Ludwigshafen, FRG.

Best-price.com. 2007. [Search results for butter flavoring] Internet address: <http://www.best-price.com/procSystem.showProductSearchByUserSearch,,0,butter%20flavoring.html>. Last accessed on January 25, 2007.

Bratovanova, P. 2001. To the issue of formation of acetoin and diacetyl in dough part manufactured products. *Biotechnol Biotechnol Equip*, 15(2):124-127. Abstract from CAPLUS 2002:109581.

Bunge Oils Inc. 2004. Nutrition information and ingredient declaration. Internet address: <http://www.bungefoods.com/LiquidButterContent.htm>. Last accessed on January 25, 2007.

CCRIS (Chemical Carcinogenesis Research Information System). 1995. 2,3-Butanedione. CCRIS Record No. 827. Record last revised on March 3, 1995. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov>. Last accessed on November 13, 2006.

ChemExper.com. 2006. Internet address: <http://www.chemexper.com/>. Last accessed on December 18, 2006.

ChemIDplus. 2004a. Diacetyl; RN: 431-03-8. Search at Internet address: <http://chem.sis.nlm.nih.gov/chemidplus/>. Last accessed on November 13, 2006.

ChemIDplus. 2004b. Acetoin; RN: 513-86-0. Search at Internet address: <http://chem.sis.nlm.nih.gov/chemidplus/>. Last accessed on November 13, 2006.

Country Kitchen SweetArt, Inc. Undated. [Search results for butter flavoring.] Internet address: <http://www.countrykitchensa.com/catalog/searchresults.aspx?Description=butter+flavoring>. Last accessed on January 17, 2007.

Csiba, A., Szentgyorgyi, M., and Lombai, G. 1999. Identification of health hazardous substances from roasted coffee. *Ellemezési Ipar*, 53(2):37-41. Abstract from CAPLUS 1999:243155.

- Elmore, J.S., Mottram, S.S., and Hierro, E. 2001. Two-fibre solid-phase microextraction combined with gas chromatography-mass spectrometry for the analysis of volatile aroma compounds in cooked pork. *J Chromatogr A*, 905(1-2):233-240. Abstract from CAPLUS 2000:891101.
- Faust, B.C., Powell, K., Rao, C. J., Anastasio, C. 1997. Aqueous-phase photolysis of biacetyl (an  $\alpha$ -dicarbonyl compound): A sink for biacetyl, and a source of acetic acid, peroxyacetic acid, hydrogen peroxide, and the highly oxidizing acetylperoxyl radical in aqueous aerosols, fogs, and clouds. *Atmos Environ*, 31(3):497-510. Abstract from CAPLUS 1997:40185.
- Fedan, J.S., Dowdy, J.A., Fedan, K.B., and Hubbs, A.F. 2006. Popcorn worker's lung: *in vitro* exposure to diacetyl, an ingredient in microwave popcorn butter flavoring, increases reactivity to methacholine. *Toxicol Appl Pharmacol*, 215(1):17-22.
- Friedrich, J.E., and Acree, T.E. Undated. Gas chromatography olfactometry (GC/O) of dairy products. Internet address: [http://www.nysaes.cornell.edu/fst/faculty/acree/nizo\\_tea.pdf](http://www.nysaes.cornell.edu/fst/faculty/acree/nizo_tea.pdf).
- Food History, <http://www.kitchenproject.com/history/popcorn.htm>
- Fujioka, K., and Shibamoto, T. 2006. Determination of toxic carbonyl compounds in cigarette smoke. *Environ Toxicol*, 21(1):47-54. Abstract from PubMed 16463255.
- Greenberg, J.P., Friedli, H., Guenther, A.B., Hanson, D., Harley, P., and Karl, T. 2006. Volatile organic emissions from the distillation and pyrolysis of vegetation. *Atmos Chem Phys*, 6(1):81-91. Abstract from CAPLUS 2006:174580.
- Hansen, J.T., and Hoffa, J.P. 2006 lett. Letter addressed to The Honorable Elaine L. Chao, Secretary of Labor. Re: Petition for an OSHA emergency temporary standard for diacetyl. Sent via facsimile, dated July 26, 2006. Internet address: [http://www.worksafe.org/images/contentEdit/docs/2006-07-26\\_UFCW-IBT\\_diacetyl\\_Petition%5B4pps%5D.pdf](http://www.worksafe.org/images/contentEdit/docs/2006-07-26_UFCW-IBT_diacetyl_Petition%5B4pps%5D.pdf).
- Hayasaka, Y., and Bartowsky, E.J. 1999. Analysis of diacetyl in wine using solid-phase microextraction combined with gas chromatography-mass spectrometry. *J Agric Food Chem*, 47(2):612-617. Abstract from PubMed 10563940.
- Hot Sauce World. Undated. Marinades for steak, chicken, and pork – meat rubs, oils... Internet address: <http://www.hotsauceworld.com/cookingaids.html>. Last accessed on January 25, 2007.
- Houeto, P., Borron, S.W., Marliere, F., Baud, F.J., and Levillain, P. 2001. Development of a method for measuring volatile organic compounds in the blood of fire victims using 'purge and trap' gas chromatography. *Indoor Built Environ*, 10(2):62-69. Abstract from CAPLUS 2001:735074.
- HSDB (Hazardous Substances Database). 2002. Diacetyl. HSDB No. 297. Record last revised on October 16, 2002. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on November 13, 2006.
- HSDB. 2005a. Acetoin. HSDB No. 974. Record last revised on November 14, 2005. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on December 4, 2006.

HSDB. 2005b. Ozone. HSDB No. 717. Profile last revised on June 24, 2005. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on December 26, 2006.

Hubbs, A.F., Battelli, L.A., Goldsmith, W.T., Porter, D.W., Frazer, D., Friend, S., Schwegler-Berry, D., Mercer, R.R., Reynolds, J.S., Grote, A., Castranova, V., Kullman, G., Fedan, J.S., Dowdy, J., and Jones, W.G. 2002. Necrosis of nasal and airway epithelium in rats inhaling vapors of artificial butter flavoring. *Toxicol Appl Pharmacol*, 185:128-135.

Hubbs, A.F., Battelli, L.A., Mercer, R.R., Kashon, M., Friend, S., Schwegler-Berry, D., and Goldsmith, W.T. 2004 abstr. Inhalation toxicity of the flavoring agent, diacetyl (2,3-butanedione), in the upper respiratory tract of rats. Abstract No. 2127. *Toxicol Sci*, 78(Suppl. 1):438-439.

International Flavor and Fragrances, Inc. 2007. Our products: Dairy. Internet address: <http://www.iff.com/internet/nsf/ProdDairy!OpenForm>. Last accessed on January 25, 2007.

iPrepare.com. 2006. Food bars – Emergency supplies. Internet address: <http://www.iprepare.com/food-bars.html>. Last accessed on January 25, 2007.

Jewell, A.M., and Seaman, T. [assignee: Anne M. Jewell]. 1994 pat. Process for producing a fat-substitute bakery dough and the fat substitute bakery products. U.S. Patent No. 821735. Filing date: January 15, 1992. (Patent issued on September 6, 1994). Internet address: <http://www.patentstorm.us/patents/5344663-fulltext.html>. Last accessed on January 25, 2007.

Kanwal, R. 2003 lett. Letter to Frank Morrison, Nebraska Popcorn, Clearwater, Nebraska, regarding findings from February 2002 HHE at plant dated July 2, 2003. HETA 2002-0089. Internet address: <http://www.cdc.gov/niosh/hhe/reports/pdfs/2002-0089-letter.pdf>.

Kanwal, R. and Kullman, G. 2004. NIOSH Health Hazard Evaluation Report. HETA 2003-0112-2949. ConAngra Snack Foods, Marion, OH. Internet address: <http://www.cdc.gov/niosh/hhe/reports/pdfs/2003-0112-2949.pdf>.

Kanwal, R., and Martin, S.B., Jr. 2003 lett. Letter to Keith Heuermann, B.K. Heuermann Popcorn, Inc., Phillips, Nebraska, regarding findings from August 2001 HHE at plant dated May 13, 2003. HETA 2001-0517. Internet address: <http://www.cdc.gov/niosh/hhe/reports/pdfs/2001-0517-letter.pdf>.

Kanwal, R., Boylstein, R.J., and Piacitelli, C. 2004. NIOSH Health Hazard Evaluation Report. HETA 2001-0474-2943. American Pop Corn Company, Sioux City, Iowa. Internet address: <http://www.cdc.gov/niosh/hhe/reports/pdfs/2001-0474-2943.pdf>.

Kanwal, R., Kullman, G., Fedan, K., and Kreiss, K. 2006b. NIOSH Health Hazard Evaluation Report. HETA 2000-0401-2991. Gilster-Mary Lee Corporation, Jasper, Missouri. Internet address: <http://www.cdc.gov/niosh/hhe/reports/pdfs/2000-0401-2991.pdf>.

Kanwal, R., Kullman, G., Piacitelli, C., Boylstein, R., Sahakian, N., Martin, S., Fedan, K., and Kreiss, K. 2006a. Evaluation of flavorings-related lung disease risk at six microwave popcorn plants. *J Occup Environ Med*, 48(2):149-157.

Karl, T., Harren, F., Warneke, C., de Gouw, J., Grayless, C., and Fall, R. 2005. Senescing grass crops as regional sources of reactive volatile organic compounds. *J Geophys Res [Atmospheres]*, 110(D15):D15302/1-D15302/11. Abstract from CAPLUS 2005:1082391.

- Kong-Chan, J.L.Y., Hellyer, J.A., and Tafuri, M.W. [assignee: The Procter and Gamble Company, Cincinnati, OH]. 1991. Simulated cheese analogs with reduced animal fat and calories. U.S. Patent No. 5061405. Filing date: October 30, 1990. Internet address: <http://www.wikipatents.com/5061504.html>. Last accessed on January 25, 2007.
- Kovacic, P., and Cooksy, A.L. 2005. Role of diacetyl metabolite in alcohol toxicity and addiction via electron transfer and oxidative stress. *Arch Toxicol*, 79(3):123-128. Abstract from CAPLUS 2005:272427.
- Kreiss, K., Gomaa, A., Kullman, G., Fedan, K., Simoes, E.J., and Enright, P.L. 2002. Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. *N Engl J Med*, 347(5):330-338.
- Lee, S.-R., Macku, C., and Shibamoto, T. 1991. Isolation and identification of headspace volatiles formed in heated butter. *J Agric Food Chem*, 39:1972-1975.
- Louhelainen, K., Kangas, J., Veijanen, A., and Viilos, P. 2001. Effect of *in situ* composting on reducing offensive odors and volatile organic compounds in swineeries. *AIHAJ*, 62(2):159-167. Abstract from PubMed 11331987.
- McCarthy, S.L. 1995. Analysis of diacetyl and 2,3-pentanedione in beer by HPLC with fluorometric detection. *J Am Soc Brew Chem*, 53(4):178-181.
- Morgan, L., Flake, G., Kirby, P. J., Burka, L. T., Kleeberger, S. R., Garantzotis, S., Germolec, D. R. and Palmer, S. M. 2006. Respiratory tract toxicity of diacetyl in C57BL/6 mice. Society of Toxicology 45<sup>th</sup> Annual Meeting, San Diego, CA, March, 2006. *Toxicol Sci Supplement* 90(1):210.
- Northwest Indiana Times. 2006. Packager looking for business to start poppin'. Internet address: <http://www.nwitimes.com/articles/2006/08/10/business/business/99c43cc3a95431f9862571c50075f068.prt>. Last accessed on January 24, 2007.
- NTP (National Toxicology Program). 1994. 2,3-Butanedione. CAS Number 431-03-8. Chemical background document prepared for NCI by Technical Resources, Inc. under Contract No. N01-CP-56019. November 1993; revised January 1994. Available from the NTP web site at Internet address: [http://ntp.niehs.nih.gov/ntp/htdocs/Chem\\_Background/ExSumPdf/431-03-8.pdf](http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/431-03-8.pdf). Last accessed on November 13, 2006.
- Opdyke, D.L.J. 1979. Monographs on fragrance raw materials: Acetoin. *Food Cosmet Toxicol*, 17:509-533.
- Otsuka, M., Mine, T., Ohuchi, K., and Ohmori, S. 1996. A detoxication route for acetaldehyde: metabolism of diacetyl, acetoin, and 2,3-butanediol in liver homogenate and perfused liver of rats. *J Biochem (Tokyo)*, 119(2):246-251.
- Parinet, A.J., and Von Essen, S. 2002. Rapidly progressive, fixed airway obstructive disease in popcorn workers: A new occupational pulmonary illness? [Letter to the Editor] *J Occup Environ Med*, 44(3):216-218.
- Pendergrass, S.M. 2004. Method development for the determination of diacetyl and acetoin at a microwave popcorn plant. *Environ Sci Technol*, 38(3):858-861.
- QualityExtracts.com. Undated. Butter extract. Internet address: <http://www.qualityextracts.com/butter.html>. Last accessed on January 17, 2007.



- Rao, X., Kobayashi, R., White-Morris, R., Spaulding, R., Frazey, P., and Charles, M.J. 2001. GC/ITMS measurement of carbonyls and multifunctional carbonyls in PM<sub>2.5</sub> particles emitted from motor vehicles. *J AOAC Int*, 84(3):699-705. Abstract from PubMed 11417634.
- Registry. 1984. RN 431-03-8. Database available from the American Chemical Society on STN International. Record entered STN on November 16, 1984.
- Rodrigues, J.A., Barros, A.A., and Rodrigues, P.G. 1999. Differential pulse polarographic determination of alpha-dicarbonyl compounds in foodstuffs after derivatization with *o*-phenylenediamine. *J Agric Food Chem*, 47(8):3219-3222. Abstract from PubMed 10552634.
- Rome, L.H., and Miller, J. 1980. Butanedione treatment reduces receptor binding of a lysosomal enzyme to cells and membranes. *Biochem Biophys Res Commun*, 92(3):986-993.
- RTI (Research Triangle Institute). 1997. Final Report on Disposition and Excretion of [<sup>14</sup>C]2,3-Butadione in Male Rats Following Oral Administration. RTI Study No. 64U-6855. RTI Protocol No. RTI-6855-06. Report from RTI, Research Triangle Park, NC, to National Institute of Environmental Health Sciences, Research Triangle Park, NC.
- Sahakian, N., Choe, K., Boylstein, R., and Schleiff, P. 2003. NIOSH Health Hazard Evaluation Report. HETA 2002-0408-2915. Agrilink Foods Popcorn Plant, Ridgway, Illinois. Internet address: <http://www.cdc.gov/niosh/hhe/reports/pdfs/2002-0408-2915.pdf>.
- Schauer, J.J., Kleeman, M.J., Cass, G.R., and Simoneit, B.R.T. 2002a. Measurement of emissions from air pollution sources. 4. C<sub>1</sub>-C<sub>27</sub> organic compounds from cooking with seed oils. *Environ Sci Technol*, 36(4):567-575. Abstract from PubMed 11883419.
- Schauer, J.J., Kleeman, M.J., Cass, G.R., and Simoneit, B.R.T. 2002b. Measurement of emissions from air pollution sources. 5. C<sub>1</sub>-C<sub>32</sub> organic compounds from gasoline-powered motor vehicles. *Environ Sci Technol*, 36(6):1169-1180. Abstract from PubMed 11944666.
- Schauer, J.J., Kleeman, M.J., Cass, G.R., Simoneit, B.R.T. 1999a. Measurement of emissions from air pollution sources. 1. C<sub>1</sub> through C<sub>29</sub> organic compounds from meat charbroiling. *Environ Sci Technol*, 33(10):1566-1577. Abstract from CAPLUS 1999:226376.
- Schauer, J.J., Kleeman, M.J., Cass, G.R., Simoneit, B.R.T. 1999b. Measurement of emissions from air pollution sources. 2. C<sub>1</sub> through C<sub>30</sub> organic compounds from medium duty diesel trucks. *Environ Sci Technol*, 33(10):1578-1587. Abstract from CAPLUS 1999:229245.
- Schmalfuss, H. 1950. Diacetyl, an aroma component of tobacco smoke. *Rev Intern Tabacs*, 25:89. Abstract from TOXCENTER 1951:487.
- Science News, 2005. Internet address: [http://pubs.acs.org/subscribe/journals/esthag-w/2005/nov/science/rr\\_popcorn.html](http://pubs.acs.org/subscribe/journals/esthag-w/2005/nov/science/rr_popcorn.html). Last accessed on January 23, 2007.
- Shah, Y.C. 2006. Diacetyl. Internet address: <http://www.osha.gov/dts/sltc/methods/partial/t-pv2118/t-pv2118.html>. Last accessed on November 21, 2006.
- Snow, R.H. 1975. Investigation of odor control in the rendering industry. *Ind Odor Technol Assess*, 147-174. Abstract from CAPLUS 1876:154863.
- Spicer, C.W., Holdren, M.W., Smith, D.L., Hughes, D.P., and Smith, M.D. 1992. Chemical composition of exhaust from aircraft turbine engines. *J Eng Gas Turbines Power*, 114(1):111-117. Abstract from CAPLUS 1992:180164.



Sunesson, A.L., Gullberg, J., and Blomquist, G. 2001. Airborne chemical compounds on dairy farms. *J Environ Monit*, 3(2):210-216. Abstract from PubMed 11354733.

Sweet Celebrations Inc. 2003. Flavor Mill flavors. Internet address: <http://www.sweetc.com.flavors.htm>. Last accessed on January 25, 2007.

Sysco.com. 2006. Imperial Butter-It. Internet address: <http://www.sysco.com/products/productpage.asp?prodID=129&ctID=50&ptID=1>. Last accessed on January 17, 2007.

Taubert, D., Lazar, A., and Schomig, E. 2002 lett. Bronchiolitis in popcorn-factory workers. [Letter to the Editor] *N Engl J Med*, 347(24):1980-1982.

Tolvanen, O.K., Hanninen, K.I., Veijanen, A., and Villberg, K. 1998. Occupational hygiene in biowaste composting. *Waste Manag Res*, 16(6):525-540. Abstract from CAPLUS 1998:802516.

Turrisi, L. [assignee: Lever Brothers Company, New York, NY]. 1985 pat. Low calorie syrup. U.S. Patent No. 4528205. Filing date: January 9, 1984. Internet address: <http://www.freepatentsonline.com/4528205.html>. Last accessed on January 25, 2007.

USEPA IUR, 2002. United States Environmental Protection Agency, Inventory Update Reporting (IUR) database. Internet address: <http://www.epa.gov/opptintr/iur/tools/data/2002-vol.htm>. Last accessed on April 16, 2007.

Vogelzang, P.F., van der Gulden, J.W., Folgering, H., Kolk, J.J., Heederik, D., Preller, L., Tielen, M.J., and van Schayck, C.P. 1998. Endotoxin exposure as a major determinant of lung function decline in pig farmers. *Am J Respir Crit Care Med*, 157(19):15-18. Abstract from PubMed 9445272.

webexhibits.org. 2006. Methyl ketones. Internet address: <http://webexhibits.org/butter/compounds-methylKetones.html>. Last accessed on December 18, 2006.

Wilkins, K., Nielsen, E.M., and Wolkoff, P. 1997. Patterns in volatile organic compounds in dust from moldy buildings. *Indoor Air*, 7(2):128-134. Abstract from CAPLUS 1997:438167.

Yaylayan, V.A., Haffenden, L., Chu, F.L., and Wnorowski, A. 2005. Oxidative pyrolysis and postpyrolytic derivatization techniques for the total analysis of maillard model systems: Investigation of control parameters of maillard reaction pathways. *Ann NY Acad Sci*, 1043:41-54.

### 13.0 References Considered But Not Cited

Furihata, C., and Matsushima, T. 1995. *In vivo* short-term assays for tumor initiation and promotion in the glandular stomach of Fischer rats. *Mutat Res*, 339(1):15-35.

Izumo, T., Ichiki, C., Saitou, K., Otsuka, M., Ohmori, S., and Kamei, C. 1998. Effects of ethanol, acetoin, and 2,3-butanediol on EEG power spectra in conscious rats. *Biol Pharm Bull*, 21(1):29-33. Abstract from PubMed 9477165.

Nagano, M., Misumi, J., and Nomura, S. 1983. An electrophysiological study on peripheral neurotoxicity of 2,3-butanedione, 2,4-pentanedione and 2,5-hexanedione in rats (Jpn.). *Sangyo Igaku*, 25(6):471-482. Abstract from CAPLUS 1984:449780.

Nakajima, D., Ishii, R., Kageyama, S., Onji, Y., Mineki, S., Morooka, N., Takatori, K., and Goto, S. 2006. Genotoxicity of microbial volatile organic compounds. *J Health Sci*, 52(2):148-153.

Otsuka, M., Harada, N., Itabashi, T., and Ohmori, S. 1999. Blood and urinary levels of ethanol, acetaldehyde, and C4 compounds such as diacetyl, acetoin, and 2,3-butanediol in normal male students after ethanol ingestion. *Alcohol*, 17(2):119-124. Abstract from PubMed 10064379.

Roberts, D.W., York, M., and Basketter, D.A. 1999. Structure-activity relationships in the murine local lymph node assay for skin sensitization:  $\alpha,\beta$ -Diketones. *Contact Dermatitis*, 41(1):14-17. Abstract from CAPLUS 1999:442087.

Walker, R., Flanagan, R.J., Lennard, M.S., Mills, G.A., and Walker, V. 2006. Solid-phase microextraction: Investigation of the metabolism of substances that may be abused by inhalation. *J Chromatogr Sci*, 44(7):387-393. Abstract from CAPLUS 2006:777222.

Yamaguchi, T., and Nakagawa, K. 1983. Mutagenicity of and formation of oxygen radicals by trioses and glyoxal derivatives. *Agric Biol Chem*, 47(11):2461-2465. Abstract from CAPLUS 1984:30774.

### **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**

°C = degrees Celsius

µg/L = microgram(s) per liter

µg/m<sup>3</sup> = 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<sub>50</sub> = lethal concentration for 50% of test animals

LD<sub>50</sub> = 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<sup>3</sup> = 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 <sup>a</sup>

Chemical Name	CASRN	PubChem CID	Dry <sup>b</sup> popcorn	Sweet <sup>c</sup> Butter at: (°C)	Butter Flavor <sup>d</sup> Ridgway (Liq. or Paste)	QC Room Ridgway <sup>e</sup>	Mixing Room			
							Ridgway <sup>e</sup>	Sioux City (Liq. or Paste) <sup>f</sup>	Sioux City (Powder)	Jasper <sup>g</sup>
Major Constituents Identified in Butter Flavoring and Found in Room Air										
Diacetyl; 2,3-Butanedione <sup>h</sup>	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 <sup>b</sup> popcorn	Sweet <sup>c</sup> Butter at: (°C)	Butter Flavor <sup>d</sup> Ridgway (Liq. or Paste)	QC Room Ridgway <sup>e</sup>	Mixing Room			
							Ridgway <sup>e</sup>	Sioux City <sup>f</sup> (Liq. or Paste)	Sioux City (Powder)	Jasper <sup>g</sup>
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 coming 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 isobutyrate]*	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 <sub>18</sub> H <sub>14</sub> O <sub>4</sub> 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 <sup>b</sup> popcorn	Sweet <sup>c</sup> Butter at: (°C)	Butter Flavor <sup>d</sup> Ridgway (Liq. or Paste)	QC Room Ridgway <sup>e</sup>	Mixing Room			
							Ridgway <sup>e</sup>	Sioux City <sup>f</sup> (Liq. or Paste)	Sioux City (Powder)	Jasper <sup>g</sup>
Ethyl propionate	105-37-3	7749			Major	NL	NL	NL	NL	NL
<b>Ethyl vinyl ether; Vinamar</b>	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
<b>Propanal; Propionaldehyde</b>	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
<b>Chemicals Identified in Room Air but NOT Butter Flavoring</b>										
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
<b>Tetradecane</b> [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
<b>Furfural; 2-Furaldehyde; 2-Hydroxymethylfuran</b>	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 <sup>b</sup> popcorn	Sweet <sup>c</sup> Butter at: (°C)	Butter Flavor <sup>d</sup> Ridgway (Liq. or Paste)	QC Room Ridgway <sup>e</sup>	Mixing Room			
							Ridgway <sup>e</sup>	Sioux City (Liq. or Paste)	Sioux City (Powder)	Jasper <sup>g</sup>
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
<b>Octanal</b>	124-13-0	454	<b>x</b>	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
<b>Butyl Cellosolve<sup>®</sup>; Ethylene glycol monobutyl ether</b>	111-76-2	8133			NL	Minor	Minor	Minor	Major	NL
C <sub>8</sub> aliphatic hydrocarbons	N/A	N/A			NL	Major	Minor	Minor	Minor	NL
C <sub>9</sub> -C <sub>16</sub> mostly branched alkanes and aliphatic hydrocarbons plus some C <sub>9</sub> -C <sub>10</sub> alkylbenzenes	N/A	N/A			NL	Major	Major	Minor	Minor	NL
<b>p-Dichlorobenzene</b>	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
<b>1-Methoxy-2-propanol; Propylene glycol monomethyl ether</b>	107-98-2	7900			NL	Major	Major	Major	Minor	NL
<b>3-Methyl-3-buten-2-one; isopropenyl methyl ketone</b>	814-78-8	13143			NL	Minor	Minor	Major	Minor	NL
<b>α-Methylstyrene (methylstyrene isomer)</b>	98-83-9	7407			NL	Major	Minor	Minor	?	NL
Nonane	111-84-2	8141	<b>x</b>	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
<b>Bromoethane; Ethyl bromide</b>	74-96-4	6332			NL	Major	?	NL	NL	NL
<b>3-Buten-2-one; Methyl vinyl ketone</b>	78-94-4	6570			NL	Minor	Major	Minor	Minor	NL
C <sub>5</sub> H <sub>10</sub> isomers	N/A	N/A			NL	NL	NL	Major	Minor	NL
C <sub>6</sub> aliphatic hydrocarbons	N/A	N/A			NL	NL	NL	Major	Major	NL
<b>N,N-Dimethylformamide</b>	68-12-2	6228			NL	Minor	Major	NL	NL	NL

Chemical Name	CASRN	PubChem CID	Dry <sup>b</sup> popcorn	Sweet <sup>c</sup> Butter at: (°C)	Butter Flavor <sup>d</sup> Ridgway (Liq. or Paste)	QC Room Ridgway <sup>e</sup>	Mixing Room			
							Ridgway <sup>e</sup>	Sioux City (Liq. or Paste)	Sioux City (Powder)	Jasper <sup>g</sup>
Dimethylstyrene; p, $\alpha$ -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
<b>Naphthalene</b>	91-20-3	931	x		NL	Minor	Minor	Minor	Minor	NL
<b>Propylene glycol methyl ether acetate; PGMEA</b>	108-65-6	7946			NL	Minor	Minor	Minor	Minor	NL
<b>1,1,1-Trichloroethane; Methyl chloroform</b>	71-53-6	6278			NL	Minor	Minor	Minor	?	NL
<b>2-Butenal; Crotonaldehyde</b> [(E)-Crotonaldehyde (123-73-9) same PubChem]	4170-30-3	447466			NL	Minor	Minor	NL	NL	NL
<b>Butyl acrylate</b>	141-32-2	8846			NL	NL	NL	Minor	Minor	NL
<b>tert-Butyl peroxide?</b> ; Di-tert-butyl peroxide	110-05-4	8033			NL	Minor	Minor	NL	NL	NL
<b>Butyronitrile</b>	109-74-0	8008			NL	NL	NL	Minor	Minor	NL
C <sub>7</sub> aliphatic hydrocarbons	N/A	N/A			NL	NL	NL	Minor	Minor	NL
<b>Chloromethane; Methyl chloride</b>	74-87-3	6327			NL	?	?	NL	NL	NL
<b>Formic acid</b>	64-18-6	284			NL	NL	NL	Minor	Minor	NL
<b>Nitromethane</b>	75-52-5	6375			NL	Minor	Minor	NL	NL	NL
<b>Carbon disulfide</b>	75-15-0	6348								
1,1,2-Trichloro-1,2,2-trifluoroethane (CFC 113)	76-13-1	6428								
<b>2-Propoxyethanol; Ethylene glycol monopropyl ether</b>	2807-30-9	17756			NL	NL	NL	Minor	Minor	NL
<b>Trichlorofluoromethane; CFC-11</b>	75-69-4	6389			NL	NL	NL	?	Minor	NL
<b>Chemicals Identified in Room Air and Questionable in Butter Flavoring</b>										
<b>Perchloroethylene; 1,1,2,2-Tetrachloroethene</b>	127-18-4	31373			Major	NL	NL	Major	Major	NL
<b>Methylene chloride; Dichloromethane</b>	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
<b>Hexanal*</b> [also a soy volatile]	66-25-1	6184	x	100	Major	Minor	Minor	Minor	Major	NL
<b>Undecane</b>	1120-21-4	14257		100	Minor	Minor	Major	Major	Major	NL
Toluene	108-88-3	1140	x		Minor	Major	Major	Major	Major	NL
<b>Ethylbenzene</b>	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 <sup>b</sup> popcorn	Sweet <sup>c</sup> Butter at: (°C)	Butter Flavor <sup>d</sup> Ridgway (Liq. or Paste)	QC Room Ridgway <sup>e</sup>	Mixing Room			
							Ridgway <sup>e</sup>	Sioux City (Liq. or Paste)	Sioux City (Powder)	Jasper <sup>g</sup>
$\alpha$ -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 <sub>7</sub> -C <sub>10</sub> aliphatic aldehydes [Straight-chain aldehydes, octanal, nonanal, and decanal are listed separately.]	N/A	N/A			Minor	Minor	Minor	NL	NL	NL
<b>Decanal</b>	112-31-2	8175	<b>x</b>	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.

<sup>a</sup> 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.

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

<sup>c</sup> 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).

<sup>d</sup> 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<sup>6</sup>; the cut-off between major and minor peaks was set at 3 x 10<sup>6</sup> (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).

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

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

<sup>g</sup> 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<sup>6</sup>. 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.

<sup>h</sup> 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<sub>2</sub>=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: <a href="#">177</a> )	<p><b>Upper respiratory tract:</b> 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 &gt;100-200 ppm (HSDB #230, 2005).</p> <p><b>Lungs:</b> Lung tumors were not induced by intratracheal dosing of hamsters, but “peribronchiolar adenomatoid 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 (<a href="#">Xi et al., 2004</a>). It may induce bronchoconstriction in human asthmatics when inhaled (<a href="#">Sanchez-Toril et al., 2000</a>).</p>
Acetic acid (64-19-7; CID: <a href="#">176</a> )	Several persons acutely exposed to high concentrations after a glacial (100%) acetic acid spill developed reactive airways dysfunction syndrome (RADS) ( <a href="#">Kern, 1991</a> ). High acute exposure in rats failed to induce RADS ( <a href="#">Ariel et al., 1998</a> ).
Acetonitrile* (75-05-8; CID: <a href="#">6342</a> )	Vapors are irritating to eyes, nose, and throat. Massive exposures induce respiratory asphyxiation due to cyanide poisoning. Rats inhaling the LC <sub>50</sub> or LC <sub>84</sub> 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 ( <a href="#">NTP TR-447, 1996</a> ).
Bromoethane; Ethyl bromide (74-96-4; CID: <a href="#">6332</a> )	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: <a href="#">447466</a> ) [(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: <a href="#">6570</a> )	Twelve inhalation exposures over 12 days caused upper airway irritation and necrosis in mice and rats; rats were more sensitive ( <a href="#">Morgan et al. [NTP], 2000</a> ).
Butyl acrylate (141-32-2; CID: <a href="#">8846</a> )	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 ( <a href="#">Miller et al., 1981a, 1981b</a> ; <a href="#">Stott and McKenna, 1985</a> ; <a href="#">Vodicka et al., 1990</a> ).
Butyl Cellosolve®; Ethylene glycol monobutyl ether; EGBE; 2-Butoxyethanol (111-76-2; CID: <a href="#">8133</a> )	Pulmonary edema was reported in acute oral poisoning of an alcoholic man ( <a href="#">Bauer et al., 1992</a> ). 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 ( <a href="#">NTP TR-484, 2000</a> ).
tert-Butyl peroxide?; Di-tert-butyl peroxide (110-05-4; CID: <a href="#">8033</a> )	Low acute inhalation as well as dermal and eye toxicity (BIBRA, 1990)
Butyric acid (107-92-6; CID: <a href="#">264</a> )	<p>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<sup>3</sup>) for 1.5 hours (Danishevskii and Monastyrskaya, 1960).</p> <p>Butyric acid may have a pathophysiological role in some tumors and obstructive lung diseases or upper respiratory tract cancers. In <i>in vitro</i> studies with cell lines from human nasopharyngeal carcinomas, Na butyrate upregulated expression of IL-6 and IL-2R<math>\alpha</math>. Elevated serum IL-6 has been associated with several human cancers, including lung cancer, and chronic lung diseases, including bronchiolitis obliterans after lung transplantation (<a href="#">Chow et al., 2003</a>; <a href="#">Lu et al., 2002</a>; <a href="#">Wang et al., 1999</a>).</p>
Butyronitrile (109-74-0; CID: <a href="#">8008</a> )	REL=22 mg/m <sup>3</sup> . Symptoms of human exposure to nitriles include bronchial tightness and respiratory distress ( <a href="#">NIOSH, 1978</a> ).

Compound (CAS & PubChem No.)	Potential for Nasal and Lung Toxicity
Chloromethane; Methyl chloride (74-87-3; CID: <a href="#">6327</a> )	May be corrosive at high concentrations producing pulmonary edema with lesions and disturbing surfactant metabolism ( <a href="#">Huguenard et al., 1975</a> ). 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 ( <a href="#">Rafnsson and Gudmundsson, 1997</a> ). 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 ( <a href="#">Eustis et al. [NTP], 1988</a> ).
Decanal (112-31-2; CID: <a href="#">8175</a> )	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 ( <a href="#">Cometto-Muniz et al., 1998</a> ).
<i>p</i> -Dichlorobenzene (106-46-7; CID: <a href="#">4685</a> )	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 ( <a href="#">Elliott et al., 2006</a> ). Also one of the most common VOCs found in indoor air and in exhaled human breath (e.g., <a href="#">Wallace et al., 1991</a> ).
<i>N,N</i> -Dimethylformamide (68-12-2; CID: <a href="#">6228</a> )	No involvement of the respiratory tract in the toxicity (HSDB #78, 2005).
Dimethyl sulfide; Methyl sulfide (75-18-3; CID: <a href="#">1068</a> )	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 <sub>50</sub> in rats (4 hour exposure) was 40,250 ppm ( <a href="#">Tansy et al., 1981</a> ).
Ethylbenzene (100-41-4; CID: <a href="#">7500</a> )/Xylenes <i>o</i> -Xylene (95-47-6; CID: <a href="#">7237</a> ). <i>m</i> -Xylene (108-38-3; CID: <a href="#">7929</a> ) <i>p</i> -Xylene (106-42-3; CID: <a href="#">16821</a> )	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 ( <a href="#">Chan et al. [NTP], 1998; NTP TR-466, 1999</a> ).
Ethyl vinyl ether; Vinamar (109-92-2; CID: <a href="#">8023</a> )	Probably not a respiratory tract irritant; 4-hour LC <sub>50</sub> in rats was >21,200 mg/m <sup>3</sup> ; 15-minute LC <sub>50</sub> in mice was 324,000 mg/m <sup>3</sup> ( <a href="#">ChemIDplus, 2004</a> ).
Formaldehyde (50-00-0; CID: <a href="#">712</a> )	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: <a href="#">284</a> )	A 13-week inhalation study in rats and mice reported squamous metaplasia and degeneration in olfactory and respiratory epithelia ( <a href="#">NTP TOX-19, 1992</a> ).
Furfural; 2-Furaldehyde; 2-Hydroxymethylfuran (98-01-1; CID: <a href="#">7362</a> )	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: <a href="#">6184</a> )	Inhalation of 2 or 10 ppm did not affect pulmonary function but did cause eye and nose discomfort and headaches in humans ( <a href="#">Ernstgard et al., 2006</a> ).
Hexanoic acid; Caproic acid (142-62-1; CID: <a href="#">8892</a> )	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]	<p>Mentioned in the literature as having induced bronchiolitis obliterans (e.g., <a href="#">Boswell and McCunney, 1995</a>), 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 (<a href="#">Malbrain et al., 1997</a>). Four years after poisoning by inhalation, a patient who had suffered pulmonary edema developed pulmonary fibrosis (<a href="#">Duong et al., 2001</a>). 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<sub>2</sub>S emission sources (e.g., <a href="#">Bates et al., 1998</a>; <a href="#">Campagna et al., 2004</a>; <a href="#">Durand and Wilson, 2006</a>; <a href="#">Legator et al., 2001</a>).</p> <p>High concentrations induced nasal lesions in rodents and anosmia and dysosmia in humans (<a href="#">Brenneman et al. [CIIT], 2002</a>). 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 (<a href="#">Dorman et al. [CIIT], 2004</a>).</p> <p><b>Note:</b> Levels of hydrogen sulfide and <math>\gamma</math>-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<sub>2</sub>S (<a href="#">Buttery et al., 1997</a>).</p>
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 ( <a href="#">Abdo et al., 1998</a> ; <a href="#">NTP TR-472, 1999</a> ).
Ketene; Ethenone (463-51-4; CID: 10038) [not listed in Table I but it is a thermal decomposition product of diacetyl]	<p>Acute toxicity values in several species may be found in the IDLH documentation. Death was from pulmonary edema (<a href="#">NIOSH, 1996</a>). Acute inhalation toxicity is comparable to that of phosgene and hydrogen cyanide (<a href="#">Wooster et al., 1946</a>). Repeated exposures may lead to tolerance; delayed toxicity resembles that of phosgene-emphysema and fibrosis (HSDB #633, 2005).</p> <p><b>Note:</b> Ketene (CH<sub>2</sub>=C=O) and ketene polymers are among the thermal decomposition products of diacetyl (<a href="#">Rice and Walters, 1939</a>); appears that diacetyl in sweet butter decomposes when heated above 150 °C; diacetyl levels are lower than those at 100 °C (<a href="#">Lee et al., 1991</a>). 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 [<a href="#">SKC Inc., 2007</a>] or NIOSH 2/S92 [<a href="#">OSHA, 1992</a>; <a href="#">Sigma-Aldrich Co., 1999</a>]).</p>
Limonene <i>d,l</i> -Limonene; Dipentene (138-86-3; CID: 22311) ( <i>R</i> )-(+)-Limonene; <i>d</i> -Limonene (5989-27-5; CID: 440917) ( <i>S</i> )-(-)-Limonene; <i>l</i> -Limonene (5989-54-8; CID: 439250)	<p>Exposure to 450 mg/m<sup>3</sup> (~81 ppm) for 2 hours caused decreased vital capacity in exercising human volunteers (<a href="#">Falk-Filipsson et al., 1993</a>). The sensory irritation threshold is &gt;80 ppm for humans and &gt;100 ppm for mice. Mice showed mild broncho-constriction after short exposures to either enantiomer at &gt;1,000 ppm (<a href="#">Larsen et al., 2000</a>).</p> <p><i>d</i>-Limonene is readily oxidized by ozone and its oxidation products cause significant upper airway irritation at concentrations well below NOELs of the unoxidized form (<a href="#">Wolkoff et al., 2000</a>). Mice exposed to ozone-limonene reaction products (original limonene concentration 51 ppm [~280 mg/m<sup>3</sup>]) showed both upper airway irritation and "airflow limitation that persisted for at least 45 min post exposure" (<a href="#">Rohr et al., 2002</a>).</p>
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 ( <a href="#">Spencer et al., 2002</a> ) or in rats inhaling vapors in a 2-generation reproductive study ( <a href="#">Carney et al., 1999</a> ).
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 $\alpha$ -Methylstyrene (98-83-9; CID: 7407)	Toxicity in rodents inhaling high concentrations for 9-12 days did not involve the lungs ( <a href="#">Morgan et al. [NTP], 1999</a> ).
Naphthalene (91-20-3; CID: 931)	Rat inhalation induced nasal and lung tumors [NTP bioassay] ( <a href="#">Abdo et al., 2001</a> ).
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: <a href="#">454</a> )	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: <a href="#">31373</a> )	Occupational asthma reported in humans acutely exposed to high concentrations (Boulet, 1988).
Propanal; Propionaldehyde (123-38-6; CID: <a href="#">527</a> )	Mice, guinea pigs, and rabbits that inhaled high concentrations developed fatal pulmonary edema (HSDB #1193, 2003).
Propanoic acid; Propionic acid (79-09-4; CID: <a href="#">1032</a> )	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: <a href="#">7946</a> )	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: <a href="#">17756</a> )	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: <a href="#">12389</a> )	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 <i>n</i> -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: <a href="#">6278</a> )	Inhalation for up to 90 days produced almost no toxicity in several species (HSDB #157, 2005).
Undecane (1120-21-4; CID: <a href="#">14257</a> )	Rats inhaling each of the alkanes <i>n</i> -nonane to <i>n</i> -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

## References

- Abdo, K.M., Haseman, J.K., and Nyska, A. 1998. Isobutyraldehyde administered by inhalation (whole body exposure) for up to thirteen weeks or two years was a respiratory tract toxicant but was not carcinogenic in F344/N rats and B6C3F<sub>1</sub> mice. *Toxicol Sci*, 422):135-151. Abstract from PubMed 9579026.
- Abdo, K.M., Grumbein, S., Chou, B.J., and Herbert, R. 2001. Toxicity and carcinogenicity study in F344 rats following 2 years of whole-body exposure to naphthalene vapors. *Inhal Toxicol*, 13(10):931-950. Abstract from PubMed 11696867.
- Ariel, A.P., Furlott, H.G., Chapman, K.R., Slutsky, A.S., Webster, P., Zamel, N., and Tarlo, S.M. 1998. Effect of high dose inhaled acetic acid on airway responsiveness in fischer rats. *Can Respir J*, 5(5):349-354. Abstract from PubMed 9832602.
- Bates, M.N., Garrett, N., Graham, B., and Read, D. 1998. Cancer incidence, morbidity and geothermal air pollution in Rotorua, New Zealand. *Int J Epidemiol*, 27(1):10-14. Abstract from PubMed 9563687.
- Bauer, P., Weber, M., Mur, J.M., Protois, J.C., Bollaert, P.E., Condi, A., Larcan, A., and Lambert, H. 1992. Transient non-cardiogenic pulmonary edema following massive ingestion of ethylene glycol butyl ether. *Intensive Care Med*, 18(4):250-251. Abstract from PubMed 1430593.

BIBRA (The British Industrial Biological Research Association). 1990. Di-*tert*-butyl Peroxide. Toxicity Profile. The British Industrial Biological Research Association, Carshalton, U.K., 4 pp. Abstract from TOXLINE (Secondary Source ID: RISKLINE/1990050006; entered in May 1990). Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?TOXLINE>. Last accessed on January 9, 2007.

Boswell, R.T., and McCunney, R.J. 1995. Bronchiolitis obliterans from exposure to incinerator fly ash. *J Occup Environ Med*, 37(7):850-855. Abstract from PubMed 7552470.

Boulet, L.P. 1988. Increases in airway responsiveness following acute exposure to respiratory irritants. Reactive airway dysfunction syndrome or occupational asthma? *Chest*, 94(3):476-481. Abstract from PubMed 2842114.

Brenneman, K.A., Meleason, D.F., Sar, M., Marshall, M.W., James, R.A., Gross, E.A., Martin, J.T., and Dorman, D.C. 2002. Olfactory mucosal necrosis in male CD rats following acute inhalation exposure to hydrogen sulfide: Reversibility and the possible role of regional metabolism. *Toxicol Pathol*, 30(2):200-208. Abstract from PubMed 11950163.

BUA (Beratergremium für umweltrelevante Altstoffe). 2005. Caproic acid. BUA, Vol. 241, 89 pp. Abstract from TOXCENTER 2004:151446.

Buttery, R.G., Ling, L.C., and Stern, D.J. 1997. Studies on popcorn aroma and flavor volatiles. *J Agric Food Chem*, 45(3):837-843.

Campagna, D., Kathman, S.J., Pierson, R., Inserra, S.G., Phifer, B.L. Middleton, D.C., Zarus, G.M., and White, M.C. 2004. Ambient hydrogen sulfide, total reduced sulfur, and hospital visits for respiratory diseases in northeast Nebraska, 1998-2000. *J Expo Anal Environ Epidemiol*, 14(2):180-187. Abstract from PubMed 1501549.

Canadian Centre for Occupational Health Safety. 1990. Chemical information sheet: Hexanoic acid. Canadian Centre for Occupational Health Safety, 1 p. Abstract from TOXCENTER 2002:513983.

Carney, E.W., Crissman, J.W., Liberacki, A.B., Clements, C.M., and Breslin, W.J. 1999. Assessment of adult and neonatal reproductive parameters in Sprague-Dawley rats exposed to propylene glycol monomethyl ether vapors for two generations. *Toxicol Sci*, 50(2):249-258. Abstract from PubMed 10478862.

Chan, P.C., Hasemani, J.K., Mahleri, J., and Aranyi, C. 1998. Tumor induction in f344/n rats and b6c3f1 mice following inhalation exposure to ethylbenzene. *Toxicol Lett*, 99(1):23-32.

CHE (The Collaborative on Health and the Environment). Undated. CHE Toxicant and Disease Database. Displaying diseases linked to formaldehyde (grouped by strength of evidence). Internet address: <http://database.healthandenvironment.org/index.cfm>. Last accessed on January 24, 2007.

ChemIDplus. 2004. Vinamar; RN: 109-92-2. Search at Internet address: <http://chem.sis.nlm.nih.gov/chemidplus/>. Last accessed on December 20, 2006.

Chow, K.C., Chiou, S.H., Ho, S.P., Tsai, M.H., Chen, C.L., Wang, L.S., and Chi, K.H. 2003. The elevated serum interleukin-6 correlates with the increased serum butyrate level in patients with nasopharyngeal carcinoma. *Oncol Rep*, 10(4):813-819.

Cometto-Muniz, J.E., Cain, W.S., and Abraham, M.H. 1998. Nasal pungency and odor of homologous aldehydes and carboxylic acids. *Exp Brain*



Res, 118(2):180-188. Abstract from PubMed 9580061.

Danishevskii, S.L., and Monastyrskaya, B.I. 1960. Toxicity of butyric acid (Russ.). Tr Leningr Sanit Gigien Med Inst, 62:78-84. Abstract from TOXCENTER 1962:16996.

Dorman, D.C., Struve, M.F., Gross, E.A., and Brenneman, K.A. 2004. Respiratory tract toxicity of inhaled hydrogen sulfide in Fischer-344 rats, Sprague-Dawley rats, and B6C3F<sub>1</sub> mice following subchronic (90-day) exposure. Toxicol Appl Pharmacol, 198(1):29-39. Abstract from PubMed 15207646.

Duong, T.X., Suruda, A.J., and Maier, L.A. 2001. Interstitial fibrosis following hydrogen sulfide exposure. Am J Ind Med, 40(2):221-224. Abstract from PubMed 11494351.

Durand, M., and Wilson, J.G. 2006. Spatial analysis of respiratory disease on an urbanized geothermal field. Environ Res, 101(2):238-245. Abstract from PubMed 16169550.

Elliott, L., Longnecker, M.P., Kissling, G.E., and London, S.J. 2006. Volatile organic compounds and pulmonary function in the third national health and nutrition examination survey, 1988-1994. Environ Health Perspect, 114(8):1210-1214.

Ernstgard, L., Iregren, A., Sjogren, B., Svedberg, U., and Johanson, G. 2006. Acute effects of exposure to hexanal vapors in humans. J Occup Environ Med, 48(6):573-580. Abstract from PubMed 16766921.

Eustis, S.L., Haber, S.B., Drew, R.T., and Yang, R.S. 1988. Toxicology and pathology of methyl bromide in F344 rats and B6C3F<sub>1</sub> mice following repeated inhalation exposure. Fundam Appl Toxicol, 11(4):594-610. Abstract from PubMed 3229585.

Falk-Filipsson, A., Lof, A., Hagberg, M., Hjelm, E.W., and Wang, Z. 1993. d-Limonene exposure to humans by inhalation: Uptake, distribution, elimination, and effects on the pulmonary function. J Toxicol Environ Health, 38(1):77-88. Abstract from PubMed 8421324.

Gomaa, A., Kullman, G., Fedan, K., ENLight, P., Schleiff, P., Phillips, P.E., Simoes, E., and Kreiss, K.. 2001. Appendix C. NIOSH Investigation of Gilster-Mary Lee Corporation. HETA 2000-0401. Technical Assistance to Missouri Department of Health, Interim Report, August 22, 2001. Cited by Kanwal et al. (2006).

Graham, C., Rosenkranz, H.S., and Karol, M.H. 1997. Structure-activity model of chemicals that cause human respiratory sensitization. Regul Toxicol Pharmacol, 26(3):296-306. Abstract from EMBASE 1998031306.

Harris, D.T., Sakiestewa, D., Robledo, R.F., and Witten, M. 1997. Immunotoxicological effects of JP-8 jet fuel exposure. Toxicol Ind Health, 13(1):43-55. Abstract from PubMed 9098949.

HSDB (Hazardous Substances Data Bank). 2002. Decaldehyde. HSDB No. 288. Record last updated on November 8, 2002. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on January 24, 2007.

HSDB. 2002. Dimethyl sulfide. HSDB No. 356. Record last revised on November 8, 2002. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on December 5, 2006.

- HSDB. 2002. Methyl isopropenyl ketone. HSDB No. 1164. Record last updated on November 16, 2002. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on January 24, 2007.
- HSDB. 2002. Ethylene glycol monopropyl ether. HSDB No. 6499. Record last updated on May 13, 2002. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on January 24, 2007.
- HSDB (Hazardous Substances Database). 2003. Isobutyraldehyde. HSDB No. 614. Record last revised on January 24, 2003. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on January 11, 2007
- HSDB. 2003. Propionaldehyde. HSDB No. 1193. Record last updated on February 14, 2003. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on January 24, 2007.
- HSDB. 2003. Octylaldehyde. HSDB No. 5147. Record last updated on January 24, 2003. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on January 24, 2007.
- HSDB. 2005. Acetonitrile. HSDB No. 42. Record last revised on June 24, 2005. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on December 5, 2006.
- HSDB. 2005. Carbon Disulfide. HSDB No.52. Record last revised on June 24, 2005. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on January 11, 2007.
- HSDB. 2005. Dichloromethane. HSDB No. 66. Record last updated on June 24, 2005. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on January 24, 2007.
- HSDB. 2005. *N,N*-Dimethylformamide. HSDB No. 78. Record last revised on June 24, 2005. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on January 9, 2007.
- HSDB. 2005. 1,1,1-Trichloroethane. HSDB No. 157. Record last revised on June 24, 2005. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on January 26, 2007.
- HSDB. 2005. Acetaldehyde. HSDB No. 230. Record last revised on June 24, 2005. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on January 23, 2007.
- HSDB. 2005. Crotonaldehyde (2-Butenal). HSDB No.252. Record last revised on June 24, 2005. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on January 8, 2007.
- HSDB. 2005. Ethyl Bromide. HSDB No.532. Record last revised on June 24, 2005. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on January 9, 2007.
- HSDB. 2005. Ketene. HSDB No. 633. Record last revised on June 24, 2005. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on January 8, 2007.
- HSDB. 2006. Nitromethane. HSDB No. 106. Record last revised on April 14, 2006. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on January 9, 2007.

- HSDB. 2006. Furfural. HSDB No. 542. Record last updated on August 30, 2006. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on January 24, 2007.
- HSDB. 2006. Propionic acid. HSDB No. 1192. Record last updated on December 20, 2006. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on January 24, 2007.
- HSDB. 2006. Hexanoic acid. HSDB No. 6813. Record last updated on December 20, 2006. Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>. Last accessed on January 24, 2007.
- Huguenard, P., Richard, A., and Desfemmes, C. 1975. Pulmonary edema due to inhalation of gas and vapors (Fr.). *Ann Anesthesiol Fr*, 16(Spec 2-3):95-99. Abstract from PubMed 9877.
- Kanwal, R., Boylstein, R., and Piacitelli, C. 2004. NIOSH Health Hazard Evaluation Repot: HETA #2001-0474-2943. American Pop Corn Company, Sioux City, Iowa.
- Kanwal, R., Kullman, G., Fedan, K., and Kreiss, K. 2006. NIOSH Health Hazard Evaluation Report: HETA #2000-0401-2991. Glister-Mary Lee Corporation, Jasper, Missouri.
- Kern, D.G. 1991. Outbreak of the reactive airways dysfunction syndrome after a spill of glacial acetic acid. *Am Rev Respir Dis*, 144(5):1058-1064. Abstract from PubMed 1952431.
- Kerns, W.D., Pavkov, K.L., Donofrio, D.J., Gralla, E.J., and Swenberg, J.A. 1983. Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. *Cancer Res*, 43(9):4382-4392. Abstract from PubMed 6871871.
- Larsen, S.T., Hougaard, K.S., Hammer, M., Alarie, Y., Wolkoff, P., Clausen, P.A., Wilkins, C.K., and Nielsen, G.D. 2000. Effects of R-(+)- and S-(-)-limonene on the respiratory tract in mice. *Hum Exp Toxicol*, 19(8):457-466. Abstract from PubMed 11125716.
- Lee, S.-R., Macku, C., and Shibamoto, T. 1991. Isolation and identification of headspace volatiles formed in heated butter. *J Agric Food Chem*, 39:1972-1975.
- Legator, M.S., Singleton, C.R., Morris, D.L., and Philips, D.L. 2001. Health effects from chronic low-level exposure to hydrogen sulfide. *Arch Environ Health*, 56(2):123-131. Abstract from PubMed 11339675.
- Lu, K.C., Jaramillo, A., Lecha, R.L., Schuessler, R.B., Aloush, A., Trulock, E.P., Mendeloff, E.N., Huddleston, C.B., Alexander, P.G., and Mohanakumar, T. 2002. Interleukin-6 and interferon-gamma gene polymorphisms in the development of bronchiolitis obliterans syndrome after lung transplantation. *Transplantation*, 74(9):1297-1302. Abstract from PubMed 12451269.
- Malbrain, M.L.N.G., Bomans, P., Rappoort, G., and Demeyer, I. 1997. Aggressive treatment, outcome and long time neurologic and pulmonary complications of lethal hydrogen sulphide (H<sub>2</sub>S) intoxication. Meeting abstract. *Crit Care*, 1(Suppl. 1):P132. Abstract available at <http://ccforum.com/content/1/S1/P132>. Last accessed on December 5, 2006.
- Miller, R.R., Ayres, J.A., Jersey, G.C., and McKenna, M.J. 1981a. Inhalation toxicity of acrylic acid. *Fundam Appl Toxicol*, 1(3):271-277. Abstract from PubMed 7184791.

- Miller, R.R., Ayres, J.A., Rampy, L.W., and McKenna, M.J. 1981b. Metabolism of acrylate esters in rat tissue homogenates. *Fundam Appl Toxicol*, 1(6):410-414. Abstract from PubMed 7185591.
- Miller, R.R., Hermann, E.A., Young, J.T., Calhoun, L.L., and Kastl, P.E. 1984. Propylene glycol monomethyl ether acetate (PGMEA) metabolism, disposition, and short-term vapor inhalation toxicity studies. *Toxicol Appl Pharmacol*, 7(3):521-530. Abstract from PubMed 6474479.
- Morgan, D.L., Mahler, J.F., Kirkpatrick, D.T., Price, H.C., O'Connor, R.W., Wilson, R.E., and Moorman, M.P. 1999. Characterization of alpha-methylstyrene vapor toxicity for B6C3F1 mice and F344 rats. *Toxicol Sci*, 47(2):187-194. Abstract from PubMed 10220856.
- Morgan, D.L., Price, H.C., O'Connor, R.W., Seely, J.C., Ward, S.M., Wilson, R.E., and Cunningham, M.C. 2000. Upper respiratory tract toxicity of inhaled methyl vinyl ketone in F344 rats and B6C3F1 mice. *Toxicol Sci*, 58(1):182-194. Abstract from PubMed 11053555.
- Narotsky, M.G., Francis, E.Z., and Kavlock, R.J. 1991 abstr. Continued evaluation of structure-activity relationships in the developmental effects of aliphatic acids in rats. *Teratology*, 43(5):433. Abstract from TOXCENTER 2002:564105.
- Narotsky, M.G., Francis, E.Z., and Kavlock, R.J. 1994. Developmental toxicity and structure-activity relationships of aliphatic acids, including dose-response assessment of valproic acid in mice and rats. *Fundam Appl Toxicol*, 22(2):251-265. Abstract from TOXCENTER 1994:140070.
- Nilsen, O.G., Haugen, O.A., Zahlsten, K., Halgunset, J., Helseth, A., Aarset, H., and Eide, I. 1988. Toxicity of n-C9 to n-C13 alkanes in the rat on short term inhalation. *Pharmacol Toxicol*, 62(5):259-266. Abstract from PubMed 3413027.
- NIOSH (National Institute for Occupational Safety and Health). 1978. Criteria for a Recommended Standard...Occupational Exposure to Nitriles. DHEW (NIOSH) Publication No. 78-212. NIOSH, U.S. Department of Health, Education, and Welfare, Cincinnati, OH, 163 pp. Abstract from TOXLINE (Secondary Source ID: NIOSH/00184106; entered in March 1990). Internet address (available via TOXNET on NLM): <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?TOXLINE>. Last accessed on January 9, 2007.
- NIOSH. 1996. Ketene. IDLH Documentation. Internet address: <http://origin.cdc.gov/niosh/idlh/463514.html>. Last updated on August 16, 1996. Last accessed on January 23, 2007.
- NTP (National Toxicology Program). 1992. NTP Technical Report on Toxicity Studies of Formic Acid (CAS No: 64-18-6) Administered by Inhalation to F344/N Rats and B6C3F1 Mice. NTP Toxicity Report Series Number 19. NIH Publication 92-3342. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC. Abstract available at <http://ntp.niehs.nih.gov/index.cfm?objectid=072CE030-D41D-EC74-BAD89399317E5524> and the full text at [http://ntp.niehs.nih.gov/ntp/htdocs/ST\\_rpts/tox019.pdf](http://ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/tox019.pdf). Both last accessed on January 11, 2007.
- NTP. 1996. Toxicology and Carcinogenesis Studies of Acetonitrile (CAS No. 75-05-8) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). Technical Report No. 447. Abstract available at Internet address: <http://ntp.niehs.nih.gov/index.cfm?objectid=070A3148-FA40-A415-245E1A1CC0AEECF4>. Last updated on October 4, 2004. Last accessed on December 11, 2006.
- NTP. 1999. Toxicology and Carcinogenesis Studies of Ethylbenzene (CAS No. 100-41-4) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). Technical Report No. 466. Abstract available at Internet address: <http://ntp.niehs.nih.gov/ntpweb/index.cfm?objectid=070A7F1A-CD44-B3EE-05596CD253F268B6>. Last updated on October 14, 2004. Last accessed on December 18, 2006.

- NTP. 1999. NTP Toxicology and Carcinogenesis Studies of Isobutyraldehyde (CAS No. 78-84-2) in F344/N Rats and B6C3F<sub>1</sub> Mice (Inhalation Studies). Technical Report No. 472, 242 pp. Abstract from PubMed 12579201.
- NTP. 2000. NTP Toxicology and Carcinogenesis Studies 2-Butoxyethanol (CAS No. 111-76-2) in F344/N Rats and B6C3F<sub>1</sub> Mice (Inhalation Studies). Technical Report No. 484, 290 pp. Abstract from PubMed 12571679.
- OSHA (Occupational Safety and Health Administration). 1992. Chemical sampling information: Ketene. Internet address: [http://www.osha.gov/dts/chemicalsampling/data/CH\\_248900.html](http://www.osha.gov/dts/chemicalsampling/data/CH_248900.html). Last updated on August 24, 1992. Last accessed on January 26, 2007.
- Pfaff, J., Parton, K., Lantz, R.C., Chen, H., Hays, A.M., and Witten, M.L. 1995. Inhalation exposure to JP-8 jet fuel alters pulmonary function and substance P levels in Fischer 344 rats. *J Appl Toxicol*, 15(4):249-256. Abstract from PubMed 7594192.
- Rafnsson, V., and Gudmundsson, G. 1997. Long-term follow-up after methyl chloride intoxication. *Arch Environ Health*, 52(5):355-359. Abstract from PubMed 9546758.
- Rengarajan, R., and Seitz, L.M. 2004. Chapter 22. Analysis of flavor compounds from microwave popcorn using supercritical fluid CO<sub>2</sub> followed by dynamic/static headspace techniques. *Food Sci Technol*, 131(Handbook of Flavor Characterization):311-334. Abstract from CAPLUS 2003:878123.
- Rice, F.O., and Walters, W.D. 1939. The thermal decomposition of diacetyl. *J Chem Phys*, 7(11):1015-1018. Abstract available at Internet address: <http://scitation.aip.org/getabs/servlet/GetabsServlet?prog=normal&id=JCPA6000007000011001015000001&idtype=cvips&gifs=yes>. Last accessed on November 17, 2006.
- Robledo, R.F., Young, R.S., Lantz, R.C., and Witten, M.L. 2000. Short-term pulmonary response to inhaled JP-8 jet fuel aerosol in mice. *Toxicol Pathol*, 28(5):656-663. Abstract from PubMed 11026600.
- Rohr, A.C., Wilkins, C.K., Clausen, P.A., Hammer, M., Nielsen, G.D., Wolkoff, P., and Spengler, J.D. 2002. Upper airway and pulmonary effects of oxidation products of (+)-alpha-pinene, d-limonene, and isoprene in BALB/c mice. *Inhal Toxicol*, 14(7):663-684. Abstract from PubMed 12122569.
- Sahakian, N., Choe, K., Boylstein, R., and Schleiff, P. 2003. NIOSH Health Hazard Evaluation Report: HETA #2002-0408-2915. Agrilink Foods Popcorn Plant, Ridgway, Illinois.
- Sanchez-Toril, F., Prieto, L., Peris, R., Perez, J.A., Millan, M., and Marin, J. 2000. Differences in airway responsiveness to acetaldehyde and methacholine in asthma and chronic bronchitis. *Eur Respir J*, 15(2):260-265. Abstract from PubMed 10706489.
- Sigma-Aldrich Co. 1999. Supelco Air Methods Guide. 2<sup>nd</sup> ed. Internet address: <http://www.sigmaaldrich.com/Graphics/Supelco/objects/6600/6501.pdf>.
- SKC Inc. 2007. Guide to OSHA/NIOSH/ASTM Air Sampling Methods. Internet address: [http://www.skcinco.com/OSHA-NIOSH/list.asp?Enter\\_Letter=K](http://www.skcinco.com/OSHA-NIOSH/list.asp?Enter_Letter=K). Last updated on January 26, 2007. Last accessed on January 26, 2007.

- Spencer, P.J., Crissman, J.W., Stott, W.T., Corley, R.A., Cieszlak, F.S., Schumann, A.M., and Hardisty, J.F. 2002. Propylene glycol monomethyl ether (PGME): Inhalation toxicity and carcinogenicity in Fischer 344 rats and B6C3F<sub>1</sub> mice. *Toxicol Pathol*, 30(5):570-579. Abstract from PubMed 12371666.
- Stott, W.T., and McKenna, M.J. 1985. Hydrolysis of several glycol ether acetates and acrylate esters by nasal mucosal carboxylesterase *in vitro*. *Fundam Appl Toxicol*, 5(2):399-404. Abstract from PubMed 3988008.
- Tansy, M.F., Kendall, F.M., Fantasia, J., Landin, W.E., Oberly, R., and Sherman, W. 1981. Acute and subchronic toxicity studies of rats exposed to vapors of methyl mercaptan and other reduced-sulfur compounds. *J Toxicol Environ Health*, 8(1-2):71-88. Abstract from PubMed 7328716.
- Terazawa, K., Mizukami, K., Wu, B., and Takatori, T. 1991. Fatality due to inhalation of dimethyl sulfide in a confined space: A case report and animal experiments. *Int J Legal Med*, 104(3):141-144. Abstract from PubMed 1911412.
- Vodicka, P., Gut, I., and Frantik, E. 1990. Effects of inhaled acrylic acid derivatives in rats. *Toxicology*, 65(1-2):209-221. Abstract from PubMed 2274966.
- Wallace, L., Nelson, W., Ziegenfus, R., Pellizzari, E., Michael, L., Whitmore, R., Zelon, H., Hartwell, T., Perritt, R., and Westerdahl, D. 1991. The Los Angeles TEAM study: Personal exposures, indoor-outdoor air concentrations, and breath concentrations of 25 volatile organic compounds. *J Expo Anal Environ Epidemiol*, 1(2):157-192. Abstract from PubMed 1824315.
- Wang, L.S., Chow, K.C., and Wu, C.W. 1999. Expression and up-regulation of interleukin-6 in oesophageal carcinoma cells by n-sodium butyrate. *Br J Cancer*, 80(10):1617-1622. Abstract from PubMed 10408408.
- Wang, S., Young, R.S., and Witten, M.L. 2001. Age-related differences in pulmonary inflammatory responses to JP-8 jet fuel aerosol inhalation. *Toxicol Ind Health*, 17(1):23-29. Abstract from PubMed 12004922.
- Wolkoff, P., Clausen, P.A., Wilkins, C.K., and Nielsen, G.D. 2000. Formation of strong airway irritants in terpene/ozone mixtures. *Indoor Air*, 10(2):82-91. Abstract from PubMed 11980106.
- Wooster, H.A., Lushbaugh, C.C., and Redeman, C.E. 1946. The inhalation toxicity of ketene and ketene dimer. *J Ind Hyg Toxicol*, 29:56-57.
- Xi, Z.G., Chao, F.H., Yang, D.F., Sun, Y.M., Li, G.X., Zhang, H.S., Zhang, W., Yang, Y.H., and Liu, H.L. 2004. The effects of DNA damage induced by acetaldehyde (Chin.). *Huan Jing Ke Xue*, 25(3):102-105. Abstract from PubMed 15327264.