

**Evaluation of
Diacetyl
for Use as a Cigarette Ingredient**

December 2005

INTRODUCTION

Diacetyl (CAS # 431-03-8) is currently used worldwide at levels below **5 ppm** in selected cigarette brands manufactured and/or distributed by Philip Morris International. This document is a review of current published toxicology information on diacetyl abstracted from online toxicity databases.

TOXICITY DATA ON UN-BURNED MATERIAL

The following information was generated from the MICROMEDEX database tool <http://csi.micromedex.com> on December 20th 2005, unless otherwise indicated.

Overview

The following information was generated from the HSDB – Hazardous Substances Data Bank, a database of MICROMEDEX Systems (<http://csi.micromedex.com>) on December 20th 2005.

Diacetyl is endogenous in humans. It is formed when pyruvate is converted to acetoin and diacetyl. It is mainly important as a flavour component due to its buttery taste; it is used in low concentrations in ice cream, baked goods, and margarine.

As a food flavouring additive, the material has been assessed under the provisions of the *Federal Food, Drug and Cosmetic Act, section 201 (s)*, by the Expert Committee of the USA Flavour and Extract manufacturer's Association (FEMA), to be generally recognized as safe (GRAS) under current conditions of use.

The Joint FAO/WHO Expert Committee on Food Additives has assessed diacetyl as presenting no safety concerns at current levels of intake when used as a flavouring agent. The daily per capita intake is estimated at 133 µg/kg bw/day in the USA and 56 µg/kg bw/day in Europe¹. It has also been defined as a flavouring substance which may be used as foodstuffs by the *Council of Europe Committee of Experts on Flavouring Substances* at an upper level of 50 mg/kg in foods.

Diacetyl is a common cosmetic ingredient.

This material appears on the list of "Permitted Additives to Tobacco Products in the United Kingdom" (Department of Health, 2003) at a maximum level permitted for inclusion in cigarettes of 0.002 % w/w tobacco.

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Non-Human Toxicity Excerpts

1. Rats given 10, 30, 90, or 540 mg/kg/day 90 days orally. Highest level caused decr in wt gain, incr in water consumption, anemia & incr leukocyte count, & incr in wt of liver,

¹ Safety evaluations of certain food additives and contaminants, WHO Food Additive Series 42: Safety evaluation of aliphatic acyclic and alicyclic alpha-diketones and related alpha-hydroxyketones <http://www.inchem.org/documents/jecfa/jecmono/v042je20.htm>

- kidneys, & adrenal & pituitary glands. Necrotic stomach lesions seen. No-effect level: 90 mg/kg. [**PEER REVIEWED**] [Colley J et al; Food & cosmet toxicol 7 (6): 571 (1969)]
2. Diacetyl was nontumorigenic in mice when admin ip. Number of injections 24. Duration of experiment 24 wk. Dosage 1.70 mg/kg or 8.40 mg/kg. [**PEER REVIEWED**] [Stoner GD et al; Cancer res 33 (12): 3069 (1973)]
 3. The max ip dose of diacetyl tolerated by five mice previously given six ip injections over a 2-wk period was 350 mg/kg. [**PEER REVIEWED**] [Stoner GD et al; Cancer Res 33: 3069 (1973) as cited in Opdyke DLJ; Food Cosmet Toxicol 17: 765 (1979)]
 4. In cultures with ascites sarcoma cells in suspension, diacetyl (1,0.1, and 0.001 mM concns); the 1 mM and 0.1 mM concns inhibited cell growth by 100 and 37%, respectively. [**PEER REVIEWED**] [Pilotti A et al; Toxicology 5: 49 (1975) as cited in Opdyke DLJ; Food Cosmet Toxicol 17: 765 (1979)]
 5. The administration of up to 1.6 g starter distillate/kg by oral intubation to pregnant mice for ten consecutive days did not affect maternal or fetal survival or nidation, and the number of fetal abnormalities was not /statistically/ significant when compared to control animals. Similar results were observed in hamsters and rats. [**PEER REVIEWED**] [Food & Drug Res Lab. Teratologic Eval of FDA 71-73. USFDA Rep No. FDABF-GRAS-152; NTIS # PB-223833 (1973) as cited in Opdyke DLJ; Food Cosmet Toxicol 17: 765 (1979)]
 6. Diacetyl, 300- 1500 mg/kg body weight by gastric intubation, to male F344 rats increased ornithine decarboxylase activity, DNA synthesis, and apparent unscheduled DNA synthesis in the pyloric mucosa 16 hr after administration. [**PEER REVIEWED**] [Furihata, C et al; Jpn J Cancer Res (Gann) 76: 809-814 (1985)]
 7. Diacetyl was weakly mutagenic in TA104 strain of Salmonella typhimurium at 6 umoles/plate. Other dicarbonyl cmpd, methyl glyoxal and kethoxal, were more potent. [**PEER REVIEWED**] [Marnett, LJ et al; Mut Res 148: 25-34 (1985)]
 8. The in vivo/in vitro UDS assay methods described here, chemicals are given to experimental animals and the induction of UDS in target organs is determined by in vitro organ culture or primary cell culture in the presence of (3)HdThd. Incorporation of (3)HdThd into DNA is measured with liquid scintillation counter or by autoradiography. ... The review shows that the induction of UDS in various organs correlated well with the induction of cancer in these organs. ... Three dicarbonyl cmpd, glyoxal, methylglyoxal and diacetyl induced apparent UDS. ... [**PEER REVIEWED**] [Furihata C, Matsushima T; Crit Rev Toxicol 17 (3): 245-77 (1987)]
 9. ... The induction of sister chromatid exchanges and endoreduplicated cells /were measured/ to determine the genotoxicity of various 1,2-dicarbonyl cmpd in Chinese hamster ovary (CHO) AUXB1 cells and human peripheral lymphocytes. The 1,2-dicarbonyls glyoxal, methylglyoxal and kethoxal induced sister chromatid exchanges and endoreduplicated cells. ... Glyoxal and kethoxal induced sister chromatid exchanges but not endoreduplicated cells in human peripheral lymphocytes. ... These in vitro results suggest that 1,2-dicarbonyls may play an important role in the genotoxicity of some foods and beverages. [**PEER REVIEWED**] [Tucker JD et al; Mutat Res 224 (2): 269-79 (1989)]

The following information was generated from the RTECS – Registry of Toxic Effects of Chemical Substances, a database of MICROMEDEX Systems (<http://csi.micromedex.com>) on December 20th 2005.

Health hazard data

Acute toxicity

LD50/LC50 - LETHAL DOSE/CONC 50% KILL

Rat

LD50 - ROUTE: Intraperitoneal; DOSE: 400 mg/kg [Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. (7,571,1969)]

TOXIC EFFECTS:

Behavioral - Convulsions or effect on seizure threshold

Behavioral - Change in motor activity (specific assay)

Vascular - Regional or general arteriolar or venous dilation

LD50 - ROUTE: Oral; DOSE: 3000 mg/kg ['Vrednie chemichescie veshestva, galogen i kislorod sodergashie organicheskie soedinenia'. (Hazardous substances. Galogen and oxygen containing substances), Bandman A.L. et al., Chimia, 1994. (-,448,1994)]

LD50 - ROUTE: Oral; DOSE: 1580 mg/kg [Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. (2,327,1964)]

TOXIC EFFECTS:

Behavioral - Somnolence (general depressed activity)

Behavioral - Convulsions or effect on seizure threshold

Rabbit

LD50 - ROUTE: Skin; DOSE: >5 gm/kg [Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. (17,765,1979)]

Guinea Pig

LD50 - ROUTE: Oral; DOSE: 990 mg/kg [Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. (2,327,1964)]

TOXIC EFFECTS:

Behavioral - Ataxia

Behavioral - Coma

Lung, Thorax, or Respiration - Dyspnea

Irritation

SKIN - STANDARD DRAIZE TEST

Rabbit

ROUTE: Skin; DOSE: 500 mg/24H; REACTION: Moderate [Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. (17,765,1979)]

Genetic effects

UNSCHEDULED DNA SYNTHESIS

Rat

ROUTE: oral; DOSE: 1500 mg/kg [Japanese Journal of Cancer Research. (Elsevier Science Pub. BV, POB 211, 1000 AE Amsterdam, Netherlands) V.76- 1985- (76,809,1985)]

MUTATIONS IN MICROORGANISMS

Bacteria - S Typhimurium

DOSE: 1 mg/plate (+S9) [Mutation Research. (Elsevier Science Pub. B.V., POB 211, 1000 AE Amsterdam, Netherlands) V.1- 1964- (67,367,1979)]

Other multiple dose toxicity data

Rat

TDL_o - ROUTE: Oral; DOSE: 48600 mg/kg/90D intermittent [Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. (7,571,1969)]

TOXIC EFFECTS:

Liver - Changes in liver weight

Endocrine - Changes in adrenal weight

Blood - Pigmented or nucleated red blood cells

TDL_o - ROUTE: Oral; DOSE: 48600 mg/kg/90D intermittent ['Vrednie chemichescie veshstva, galogen I kislorod sodergashie organicheskie soedinenia'. (Hazardous substances. Galogen and oxygen containing substances), Bandman A.L. et al., *Chimia*, 1994. (-,448,1994)]

TOXIC EFFECTS:

Gastrointestinal - Ulceration or bleeding from stomach

Liver - Changes in liver weight

Kidney, Ureter, and Bladder - Changes in kidney weight

TOXICITY DATA ON BURNT MATERIAL

Data on the toxicity of diacetyl as a cigarette ingredient has been evaluated in a series of studies. The results of these studies may be found in the following references:

E.L. Carmines, 2002, "Evaluation of the Potential Effects of Ingredients Added to Cigarettes. Part I: Cigarette Design, Testing Approach and Review of Results," *Food and Chemical Toxicology*, 40:77-91. **PEER REVIEWED**

K. Rustemeier et al, 2002, "Evaluation of the Potential Effects of Ingredients Added to Cigarettes Part II. Chemical Smoke Composition," *Food and Chemical Toxicology*, 40:93 - 104. **PEER REVIEWED**

Roemer et al, 2002, "Evaluation of the Potential Effects of Flavor Ingredients Added to Cigarettes. Part 3. In Vitro Genotoxicity and Cytotoxicity," *Food and Chemical Toxicology*, 40:105-111. **PEER REVIEWED**

P.M. Vanscheeuwijck et al, 2002, "Toxicological Evaluation of Cigarettes without and with the Addition of Flavor Ingredients to the Tobacco. Part 4. Subchronic Inhalation Toxicity," *Food and Chemical Toxicology*, 40:113-131. **PEER REVIEWED**

These studies indicate that the ingredients used in the production of cigarettes do not increase the overall toxicity of cigarette smoke.

DATA ON THE EFFECTS ON HUMAN HEALTH

The following information was generated from the HSDB – Hazardous Substances Data Bank, a database of MICROMEDEX Systems (<http://csi.micromedex.com>) on December 20th 2005.

Human Toxicity Excerpts

Probably has irritant &.../SRP: CNS depressant/ action. **[**PEER REVIEWED**]** [Sax, N.I. Dangerous Properties of Industrial Materials. 4th ed. New York: Van Nostrand Reinhold, 1975., p. 603]

Probable Routes of Human Exposure

1. Since diacetyl is a natural component of food and is used as an aroma carrier in food products(1,2), people will be exposed by inhalation and ingestion of food(SRC). **[**PEER REVIEWED**]** [(1) Merck Index; 10th ed; p.429 (1983) (2) Graedel TE; Chemical Compounds in the Atmosphere; Academic Press New York NY (1978)]
2. NIOSH (NOES Survey 1989) estimates that 3,437 workers are exposed to diacetyl in the USA(1); 1,630 of these workers are female(1). **[**PEER REVIEWED**]** [(1) NIOSH; National Occupational Exposure Survey (NOES) (1989)]

CONCLUSION

Cigarette smoking causes lung cancer, heart disease, emphysema and other serious diseases in smokers. Smokers are far more likely to develop serious diseases, like lung cancer, than non-smokers. There is no "safe" cigarette. Government health warnings about smoking apply to all cigarettes, regardless of the ingredients added, including those containing only tobacco and paper.

While Philip Morris International has not conducted human studies on the health effects of ingredients used in cigarette manufacture, studies have been conducted using scientifically accepted in vitro and in vivo toxicity assays with various ingredient mixtures (see Toxicity Data on Burnt Material above). These studies show there is no meaningful difference in the composition or toxicity of smoke when the smoke from cigarettes with added ingredients is compared to the smoke from cigarettes without added ingredients. These findings are supported by similar studies from the published literature. It is our scientific judgment, based on the best available data, that diacetyl used in our cigarettes does not increase the overall toxicity of cigarette smoke.