Evaluation of
Acetoin
for Use as a Cigarette Ingredient

October 2006

INTRODUCTION

Acetoin (CAS # 513-86-0) is currently used worldwide at levels up to **100 ppm** by Philip Morris International in selected roll your own or conventional cigarette brands manufactured and/or distributed by Philip Morris International. This document is a review of the published toxicology information on acetoin 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 October 30th 2006, unless otherwise indicated.

Overview

Acetoin is a ketone that is found in a slightly yellow liquid form or in crystalloid form. It has a buttery odour. Acetoin is commonly used as a flavour in butter, milk, yoghurt and strawberry.

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 acetoin as presenting no safety concerns at current levels of intake when used as a flavouring agent. The daily intake is estimated at 29 µg/kg bw/day in the USA and 46 µg/kg bw/day in Europe[1]. It has also been defined as a flavouring substance which may be used as foodstuffs by the *Council of Euro*pe Committee of Experts on Flavouring Substances at an upper level of 50 mg/kg in foods.

Acetoin 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 0.15 % w/w tobacco.

The following information was generated from the HSDB – Hazardous Substances Data Bank, a database of MICROMEDEX Systems (http://csi.micromedex.com) on October 30th 2006.

Non-Human Toxicity Excerpts

1. /Laboratory animals: Acute Exposure/ The toxicity of acetoin in rats was investigated and compared to ethyl-alcohol. Small (unspecified) amounts of a 30% acetoin solution or ethyl-alcohol were injected ip into rats until the loss of the righting reflex or until respiratory failure. In another series, 5 mg/kg methyl-alcohol was injected, followed by acetoin until respiratory failure was induced. Blood was drawn and analyzed for the compounds. With loss of righting reflex, acetoin ranged from 227 to 251 mg percent with an average of 235 mg. Ethyl-alcohol ranged from 288 to 312 mg with an average of 300

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

mg. Acetoin intoxication resembled ethyl-alcohol intoxication. Upon respiratory failure, acetoin concentrations ranged from 742 to 770 mg percent with an average of 754 mg. Ethyl-alcohol ranged from 900 to 952 mg percent with an average of 920 mg. With combined administration, the concentrations of acetoin and ethyl-alcohol in the blood were additive in effect. The author concludes that acetoin is 1.4 times more intoxicating than ethyl-alcohol. [**PEER REVIEWED**] [Greenberg LA; J Pharmacol Exp Ther 77: 194-197 (1943)]

2. /Laboratory animals: Acute Exposure/ The effect of uremic toxins on oxygen consumption (OC) was studied. Male Sprague-Dawley-rats were injected intraperitoneally with acetoine. Rats were starved before and after administration of chemicals. OC was measured between 1 and 6 hours after treatment using a diaferometer or a digital respirometer. ...In-vitro OC of rat diaphragma and liver slices was measured in a Warburg apparatus using Krebs phosphate buffer at pH 7.4. ...Acetoine at 3600 mg/kg had no influence on OC. ...No decrease in blood pressure was observed. Injection of 3600 mg/kg of acetoine made them comatose for several hours. Liver slices and diaphragms from normal or uremic rats showed a normal metabolic rate. Exposure to compounds at 30 mg/dL had no influence on tissue respiration. Addition of 400 mg/dL of urea and 20 mg/dL of creatinine had no influence on OC when given alone or in combination with chemicals. [**PEER REVIEWED**] [Hohenegger M et al; Neprhron 48 (2): 154-158 (1988)]
3. /Laboratory animals: Subchronic or Prechronic Exposure/ No-untoward-effect level of acetoin was established as 3000 ppm in drinking water to rats. Levels admin were 0, 750, 3000 or 12,000 ppm for 13 wk. At 12,000 ppm body wt gain was reduced, which was assoc with redn in food & water intake. Liver wt incr. Slight anemia reported. [**PEER REVIEWED**] [Gaunt et al; Food Cosmet Toxicol 10(2) 131 (1972)]
4. /Laboratory animals: Subchronic or Prechronic Exposure/ Groups of 15 male and 15 female CFE rats were given acetoin in their drinking-water at concentrations of 0 (control), 750, 3000, or 12,000 mg/kg (equivalent to 0, 85, 330, or 1300 mg/kg bw/day). No animals died during the study, and their condition and appearance were normal. The body weights of males at 12,000 mg/kg in drinking-water decreased significantly from week 5, and at weeks 2, 6, and 13 the relative weight of the liver was statistically significantly greater in these animals than in controls. A similar effect was seen in female rats, but only after 13 weeks. Hematological examination conducted at 13 weeks showed a small (4-8%) but statistically significant ($p<0.05$) decrease in hemoglobin concentration and erythrocyte counts in animals of each sex at the high dose, but these changes were not accompanied by a decrease in hematocrit. Urinalysis and blood chemical determinations performed at the end of the study on all animals revealed no statistically significant differences between treated and control groups. Histopathological examination also revealed no adverse effects. ...The increased relative liver weights /may have been/ a reaction of the liver to an increased metabolic load resulting from the high intake of acetoin. The NOEL was 3000 ppm, equivalent to 330 mg/kg bw/day. [**PEER REVIEWED**] [WHO; Food Additive Series 42: Safety Evaluation of Aliphatic Acyclic and Alicyclic alpha-Diketones and Related alpha-Hydroxyketones (1999). Available from: http://www.inchem.org/documents/jecfa/jecmono/v042je20.htm as of February 15, 2005.]
5. /Genotoxicity/ Acetoin /(at </=4500 mg/plate)/, diacetyl, and 1,2-cyclohexanedione showed some mutagenicity in /Salmonella typhimurium/ strains TA100 and TA104. As the mutation frequencies were low and the positive results were always accompanied by negative results, the overall conclusion was that this group of substances does not induce gene mutation in bacteria in vitro. [**PEER REVIEWED**] [WHO; Food Additive

Series 42: Safety Evaluation of Aliphatic Acyclic and Alicyclic alpha-Diketones and Related alpha-Hydroxyketones (1999). Available from: http://www.inchem.org/documents/jecfa/jecmono/v042je20.htm as of February 15, 2005.]

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 October 30th 2006.

Health hazard data

Acute toxicity

LDLO/LCLO - LOWEST PUBLISHED LETHAL DOSE/CONC

Rat

LDLo - ROUTE: Subcutaneous; DOSE: 14 gm/kg [Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. (17,509,1979)]

TOXIC EFFECTS:

Peripheral Nerve and Sensation - Flaccid paralysis without anesthesia (usually neuromuscular blockage)

Behavioral - Convulsions or effect on seizure threshold

LD50/LC50 - LETHAL DOSE/CONC 50% KILL

Rat

LD50 - ROUTE: Oral; DOSE: >5 gm/kg [Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. (17,509,1979)]

Rabbit

LD50 - ROUTE: Skin; DOSE: >5 gm/kg [Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. (17,509,1979)]

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. (17,509,1979)]

Reproductive effects

Rat

TDLo - ROUTE: Oral; DOSE: 12600 mg/kg; DURATION: male 42D prior to mating [Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. (10,131,1972)]

TOXIC EFFECTS:

Paternal Effects - Testes, epididymis, sperm duct

Other multiple dose toxicity data

Rat

TDLo - ROUTE: Oral; DOSE: 66 gm/kg/13W continuous [Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. (10,131,1972)]
TOXIC EFFECTS:
Liver - Changes in liver weight
Blood - Normocytic anemia
Nutritional and Gross Metabolic - Weight loss or decreased weight gain

TOXICITY DATA ON BURNT MATERIAL

Data on the toxicity of acetoin after combustion has been evaluated in a series of studies. The results of these studies may be found in the following references:

R.R. Baker et *al*., 2004, "An overview of the effects of tobacco ingredients on smoke chemistry and toxicity", Food and chemical toxicology, 42S:53-83. **PEER REVIEWED**

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**

Gaworski et al, 1998, "Toxicological evaluation of flavor ingredients added to cigarette tobacco: 13-week inhalation exposure in rats" Inhalation Toxicology, 10:357-381. **PEER REVIEWED**

Gaworski et al, 1999, "Toxicological evaluation of flavor ingredients added to cigarette tobacco: skin painting bioassay of cigarette smoke condensate in SENCAR mice" Toxicology, 139 1-17. **PEER REVIEWED**

These studies indicate that 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 October 30th 2006.

Human Toxicity Excerpts

/Other toxicity information/ Diacetyl and acetoin are endogenous in humans. They are formed when pyruvate is converted to acetoin and diacetyl by pyruvate decarboxylase. Mean fasting blood concentrations of approximately 100 ug acetoin per 100 mL blood have been reported. [**PEER REVIEWED**] [WHO; Food Additive Series 42: Safety Evaluation of Aliphatic Acyclic and Alicyclic alpha-Diketones and Related alpha-Hydroxyketones (1999). Available from: http://www.inchem.org/documents/jecfa/jecmono/v042je20.htm as of February 15, 2005.]

Probable Routes of Human Exposure

Occupational exposure to acetoin may occur through inhalation and dermal contact with this compound at workplaces where acetoin is produced or used. The general population may be exposed to acetoin via inhalation of perfume and fragrance vapors, dermal contact, and ingestion of food products containing this compound. (SRC) [**PEER REVIEWED**]

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 acetoin used in our cigarettes does not increase the overall toxicity of cigarette smoke.