Evaluation of

Phenylacetic Acid

For Use as an Ingredient in Tobacco Products

March 2009

INTRODUCTION

Phenylacetic acid (CAS # 103-82-2) is currently used worldwide at levels up to **100 ppm** in selected brands of tobacco products manufactured and/or distributed by Philip Morris International, including cigarettes and fine-cut tobacco. This document is a review of the published toxicology information on phenylacetic acid abstracted from online toxicity databases.

Overview[a]

The following information was generated from the MICROMEDEX database tool http://csi.micromedex.com on March 19th 2009, unless otherwise indicated.

Phenylacetic acid occurs in Japanese peppermint oil, in neroli oil, and in traces in rose oils. It is a volatile aroma constituent of many foods (e.g., honey). It forms colourless crystals that have a honey odour. Because of its intense odour, phenylacetic acid is added to perfumes in small quantities for rounding off blossom odours[1]. Phenylacetic acid is also used in the manufacture of penicillin derivatives and as a starting material in the manufacture of synthetic perfumes and used as a common cosmetic ingredient.

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 phenethyl acetate as presenting no safety concerns at current levels of intake when used as a flavouring agent. The daily intake is estimated at 1µg/ kg bw/day in the USA and 5 µg/ kg bw/day in Europe[2]. 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 30 mg/kg in foods.

The use of phenylacetic acid on tobacco products is regulated in several countries worldwide. It is approved for use in tobacco products as an additive or flavouring in several countries with Tobacco Product Regulations, including e.g., Belgium, Croatia, Czech Republic, Egypt, Finland, France, Germany, Great Britain, Hungary, Lithuania, Macedonia, Romania, Slovak Republic, Spain, Switzerland. Apart from countries that approve its use, there is no country, regardless of the extent to which tobacco products are regulated therein, that affirmatively prohibits the use of this ingredient on tobacco products.

[a] Note: Philip Morris International shares the concerns of regulators and the public health community about the proliferation of certain cigarette brands that have a predominantly candy-like or fruity flavour and are particularly appealing to minors, and we support legislation that would ban such cigarettes. However, there is currently no consistent terminology used by regulators and the public health community to describe such cigarettes. This can lead to confusion and potential for misinterpretation. In this document, any references to flavours or "smoke aroma" or flavour perceptions such as "sweet", "fruity", etc. are not meant to describe a flavour, taste or aroma that would dominate the taste of the final product, let alone dominate it in a way that is appealing to minors. Rather, such references are only used to explain the differences and nuances between the various flavours described in this and related documents.

TOXICITY DATA ON UNBURNT MATERIAL

The following information was generated from HSDB – Hazardous Data Bank (last revision April 2008), a database of MICROMEDEX Systems (http://csi.micromedex.com) on March 19th 2009.

Non-human Toxicity Excerpts

1. /LABORATORY ANIMALS: Acute Exposure/ Acute oral toxicity in rats is low. Its acute effect on skin is slight irritation. /from table/ [Peer reviewed] [Patty, F. (ed.). Industrial Hygiene and Toxicology: Volume II: Toxicology. 2nd ed. New York: Interscience Publishers, 1963., p. 1839]

2. /LABORATORY ANIMALS: Acute Exposure/ In study of acute effects in mice, ip injection of 300 mg/kg proved toxic; 11 of 15 experimental animals died. Time to death varied from 10 min to 10 days. [Peer reviewed] [National Research Council. Drinking Water & Health Volume 1. Washington, DC: National Academy Press, 1977., p. 754]

3. /LABORATORY ANIMALS: Chronic Exposure or Carcinogenicity/ /It is/ reported that phenylacetic acid did not promote tumor formation when... given to rabbits iv and sc for 40 days. [Peer reviewed] [National Research Council. Drinking Water & Health Volume 1. Washington, DC: National Academy Press, 1977., p. 754]

4. /LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ ... the possible embryotoxicity of ...and phenylacetic acid /was studied/ in the postimplantation whole embryo culture system. Day 10 rat embryos (4 or 8 somites) were cultured in rat serum to which tested compounds were added. After 26 hr of culture, embryos were scored morphologically. ... and /phenylacetic acid/ induced dose-related embryotoxicity above 0.3 mg/mL. These results suggest a possible role for PKU-related phe-metabolites in the induction of congenital malformations. [Peer reviewed] [Hamers AE et al; Reprod Toxicol 5 (3): 266 (1991)]

5. /LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ In teratogenic study with rats, administration of phenylacetic acid on 12th day of embryogenesis affected body weight, retarded skeletal ossification, and caused embryos to be resorbed at twice rate of controls. Dosage was 0.2% of LD50, or 3.2 mg/kg. [Peer reviewed] [National Research Council. Drinking Water & Health Volume 1. Washington, DC: National Academy Press, 1977., p. 754]

6. /LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ The effects of phenylacetate (PA) on fetal brain growth were examined in pregnant rats receiving a 20% casein diet with 1.0, 1.5, 2.0 or 2.5% PA. Control rats were fed the 20% casein diet ad libitum or restricted to daily consumption of 9 and 6 g. In experimental groups of rats total food intake during pregnancy decreased however the decrease was not so large as ... expected. By plotting the fetal brain weight (Y, mg) against maternal food intake (X, g/21 days) in control groups the following hyperbolic regression equation was obtained: $Y = -4243/X + 124.6$ (n: 18, $r = 0.80$, $p < 0.001$). Similar plots for excess PA rats fell below this line, indicating that prenatal fetal brain growth was impaired by an excess in PA per se specifically as well as by decreased food intake nonspecifically. Administering the excess PA diets resulted in decreases in total RNA total protein and RNA/DNA ratio in the fetal brain whereas total DNA was unchanged showing the impairment of protein synthesis not proliferation. No remarkable changes in concentration and pattern of free amino acids in maternal plasma in excess PA groups were observed. Using a relation between total food

intake during pregnancy and fetal brain weight the extent of specific and nonspecific effects of excess PA and phenylalanine on brain growth was compared. /Phenylacetate/ [Peer reviewed] [Manabe S, Ohsawa K; Tokushima J Exp Med 40 (3-4): 137-45 (1993)]

7. /ALTERNATIVE and IN VITRO TESTS/ ... Of phenylacetic acid (PEAA), phenylpyruvic acid and homogentisic acid, PEAA was found to be the most effective in inhibiting carnitine biosynthesis in rats. Following 60 min, a single intraperitoneal dose of PEAA the relative conversion rate, i. e. the hydroxylation, of tracer [Me-(3)H]butyrobetaine to [Me-(3)H]carnitine decreased from 62.2+/-6.00% to 39.4+/-5.11% (means+/-S.E.M., P<0.01) in the liver, in the only organ doing this conversion in rats. The conversion of loading amount of unlabeled butyrobetaine to carnitine was also markedly reduced. The impaired hydroxylation of butyrobetaine was reflected by a reduced free and total carnitine levels in the liver and a reduced total carnitine concentration in the plasma. PEAA decreased the hepatic level of glutamic acid and alpha-ketoglutaric acid (alpha-KG), suggesting a mechanism for the reduced flux through the butyrobetaine hydroxylase enzyme, because alpha-KG is an obligatory co-enzyme. PubMed Abstract [Peer reviewed] [Fischer GM et al; Biochim Biophys Acta 1501 (2-3): 200-10 (2000)]

8. /ALTERNATIVE and IN VITRO TESTS/ ... In cultured /embryonic rat/ cortical neurons, cytoplasmic free calcium concentration ([Ca(2+)](i)) decreased dramatically when treatment with phenylalanine (Phe) and phenyllactic acid, while phenylacetic acid treatment immediately increased [Ca(2+)](i), which began to decrease after 3 min. PubMed Abstract [Peer reviewed] [Yu YG et al; Neurochem Res 32 (8): 1292-301 (2007)]

9. /OTHER TOXICITY INFORMATION/ /Phenylacetic acid inhibits activity of coenzyme A. [Peer reviewed] [National Research Council. Drinking Water & Health Volume 1. Washington, DC: National Academy Press, 1977., p. 754]

Human Toxicity Excerpts

1. /SIGNS AND SYMPTOMS/ Inhalation: cough, sore throat. Skin: redness. Eyes: redness. Pain [Peer reviewed] [International Program on Chemical Safety/Commission of the European Communities; International Chemical Safety Card on Phenylacetic acid (June 2006). Available from the Database Query page at: http://www.inchem.org/pages/icsc.html as of August 2, 2007.]

2. /OTHER TOXICITY INFORMATION/ Phenylacetate, a deaminated metabolite of phenylalanine, has been implicated in damage to immature brain in phenylketonuria. /Phenylacetate/ [Peer reviewed] [European Chemicals Bureau; IUCLID Dataset, Phenylacetic acid (103-82-2) (2000 CD-ROM edition). Available from the Database Query page at: http://ecb.jrc.it/esis/esis.php as of August 1, 2007]

Health Hazard Data

The following information was generated from the RTECS – Registry of Toxic Effects of Chemical Substances (last revision March 2000), a database of MICROMEDEX Systems (http://csi.micromedex.com) on March 19th 2009.

Acute toxicity
LD50/LC50 - LETHAL DOSE/CONC 50% KILL
Rat

LD50 - ROUTE: Intraperitoneal; DOSE: 1600 mg/kg [Bollettino Chimico Farmaceutico. (Societa Editoriale Farmaceutica, Via Ausonio 12, 20123 Milan, Italy) V.33- 1894- (112,53,1973)]

LD50 - ROUTE: Oral; DOSE: 2250 mg/kg [Voprosy Pitaniya. Problems of Nutrition. (V/O Mezhdunarodnaya Kniga, 113095 Moscow, USSR) V.1-10, 1932-41; V.11- 1952- (33(5),48,1974)]

Mouse

LD50 - ROUTE: Intraperitoneal; DOSE: 2270 mg/kg [Farmaco, Edizione Scientifica. (Casella Postale 227, 27100 Pavia, Italy) V.8-43 1953-88 For publisher information, see FRMCE8 (13,286,1958)]

LD50 - ROUTE: Oral; DOSE: 2250 mg/kg [Voprosy Pitaniya. Problems of Nutrition. (V/O Mezhdunarodnaya Kniga, 113095 Moscow, USSR) V.1-10, 1932-41; V.11- 1952- (33(5),48,1974)]

LD50 - ROUTE: Subcutaneous; DOSE: 1500 mg/kg [Archives Internationales de Pharmacodynamie et de Therapie. (Heymans Institute of Pharmacology, De Pintelaan 185, B-9000 Ghent, Belgium) V.4- 1898- (116,154,1958)]

Rabbit

LD50 - ROUTE: Skin; DOSE: >5 gm/kg [Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. (13,901,1975)]

Guinea Pig

LD50 - ROUTE: Oral; DOSE: 2250 mg/kg [Voprosy Pitaniya. Problems of Nutrition. (V/O Mezhdunarodnaya Kniga, 113095 Moscow, USSR) V.1-10, 1932-41; V.11- 1952- (33(5),48,1974)]

Irritation

EYE - STANDARD DRAIZE TEST

Rabbit

ROUTE: Eyes; DOSE: 100 mg/24H; REACTION: Moderate [National Technical Information Service. (Springfield, VA 22161) Formerly U.S. Clearinghouse for Scientific & Technical Information. (OTS0559218)]

Reproductive effects

Rat

TDLo - ROUTE: Oral; DOSE: 450 mg/kg; DURATION: female 4D of pregnancy [Voprosy Pitaniya. Problems of Nutrition. (V/O Mezhdunarodnaya Kniga, 113095 Moscow, USSR) V.1-10, 1932-41; V.11- 1952- (32,50,1973)]
TOXIC EFFECTS:
Effects on Embryo or Fetus - Fetotoxicity (except death, e.g., stunted fetus)
Specific Developmental Abnormalities - Musculoskeletal system

TOXICITY DATA ON BURNT MATERIAL

Data on the toxicity of phenylacetic acid 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**

R.A. Renne et al, 2006. "Effects of Flavoring and Casing Ingredients on the Toxicity of Mainstream Cigarette Smoke in Rats," Inhalation Toxicology, 18:685-706. **PEER REVIEWED**

CONCLUSION

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 by Philip Morris International and/or others using scientifically accepted *in vitro* and *in vivo* toxicity assays with various ingredient mixtures. These studies show there is no meaningful difference in the composition or toxicity of smoke when the smoke from cigarettes with the added ingredient is compared to the smoke from cigarettes without this added ingredient. Based on a review of current published toxicological information, it is our scientific judgment that the addition of phenylacetic acid as an ingredient, at the levels used in our brands, does not increase the overall toxicity of tobacco smoke.

References

1. **Ullmann's Encyclopedia.** *Ullmann's Encyclopedia of Industrial Chemistry : DOI:10.1002/14356007.a11_141 Article Online Posting Date: January 15, 2003*. by Wiley-VCH Verlag GmbH & Co. KGaA. **2002**.

2. **JECFA.** *WHO Food Additives Series: 50: Phenylethyl Alcohol, Aldehyde, Acid and Related Acetals and Esters and Related Substances*. **2003**.