Evaluation Summary of Ethyl Vanillin for Use as a Cigarette Ingredient

Ethyl vanillin is a synthetic analog of vanillin and does not occur in nature.[1,2] As the result of its more potent flavor, ethyl vanillin is frequently preferred to vanillin as a flavoring agent in the food, tobacco and confectionery industries.[1] Ethyl vanillin is generally recognized as safe (GRAS) by U.S. Food and Drug Administration (21 CFR § 182.60), the Flavor and Extract Manufacturers Association (FEMA No. 2464).[3]

Ethyl vanillin is readily absorbed following oral administration and metabolic by-products can be detected in the urine of humans and rats.[4,5] Toxicity studies have shown that ethyl vanillin has a low toxicity with acute lethal oral doses.[6-8] Short term and chronic animal studies have demonstrated the safety of daily exposure to ethyl vanillin in their feed.[6,8-10] There are no studies evaluating the potential teratological or reproductive effects of ethyl vanillin. Ethyl vanillin is not a sensitizer, but may be irritating to the skin.[11,12]

In vitro genotoxicity studies demonstrated that millimolar concentrations of ethyl vanillin would induce polyploidy in Chinese hamster lung cells[13,14] and sister chromatid exchanges in normal human lymphocytes. [15] Ethyl vanillin in the micromolar concentration range did not cause any genotoxic effects.[13,16,17] Ethyl vanillin was not carcinogenic or mutagenic in animal studies.[9,16,18-20]

Currently, ethyl vanillin is used worldwide at levels below 100 ppm in selected cigarette brands manufactured and/or distributed by Philip Morris USA Inc. (PM USA) and/or Philip Morris Products SA (PMP SA). Ethyl vanillin is applied directly to the tobacco as an additive, flavoring, flavoring agent, and as such, ethyl vanillin may be subject to pyrolysis-type reactions when smoked. Ethyl vanillin may also be applied to the filter as a flavoring material where it would not be subjected to pyrolysis temperatures.

As suggested by the purge and trap studies conducted by PM USA,[21] a small portion of ethyl vanillin applied to tobacco would be expected to distill at 100°C. At the higher temperatures used in the pyrolysis studies conducted by PM USA,[22] ethyl vanillin produced a number of individual chemical entities. Although no quantitation of the results was performed, 2-ethoxyphenol, or guethol, and ethyl vanillin appeared to be the most prevalent materials. The presence of guethol is not surprising, because it is often used in the manufacture of ethyl vanillin.[23,24] The results of this analysis suggest that ethyl vanillin would not be pyrolyzed and would be delivered to the smoke relatively intact.

Ethyl vanillin was a part of a PM USA testing program that was designed to evaluate the potential effects of 333 ingredients added to typical commercial blended test cigarettes on selected biological and chemical endpoints.[25-28] Three pairs of test cigarettes were produced, each containing different groups of ingredients. Ethyl vanillin was added to two pairs at target levels of below 1 ppm, 55 ppm, and 166 ppm. No significant effects were noted in cytotoxicity, mutagenic studies or in respiratory tract endpoints in 90-day rat inhalation studies. In addition, smoke chemistry studies from cigarettes containing a mixture of flavors including ethyl vanillin did not significantly alter the smoke chemistry profile compared to control cigarettes. Based on the results of these studies, the authors concluded that these ingredients (including ethyl vanillin) added to tobacco do not add significantly to the overall toxicity of cigarettes.

Currently, information is only available for tests utilizing ethyl vanillin in a mixture of ingredients applied to cigarette tobacco. Studies are ongoing to address the use of ethyl vanillin as a single ingredient. Published studies show there is no meaningful difference in the composition or toxicity of smoke from cigarettes with added ingredients (including ethyl vanillin) compared to the smoke from cigarettes without added ingredients.[25-33] Based on the best available data, the ingredients used in PM USA and/or PMP SA cigarettes do not increase the overall toxicity of cigarette smoke.

References

1. Belay, M.T. and Poole, C.F. (1993) Determination of vanillin and related flavor compounds in natural vanilla extracts and vanilla-flavored foods by thin layer chromatography and automated multiple development. *Chromatographia* 37(7-8):365-373.

2. Kahan, S. and Krueger, D.A. (1997) Liquid chromatographic method for determination of vanillin and ethyl vanillin in imitation vanilla extract (modification of AOAC official method 990.25): collaborative study. *Journal of AOAC International* 80(3):564-570.

3. Hall, R.L. and Oser, B.L. (1965) Recent progress in the consideration of flavoring ingredients under the food additives amendment. III. GRAS substances. *Food technology* 253:151-197.

4. Mamer, O.A.; Montgomery, J.A.; Deckelbaum, R.J. and Granot, E. (1985) Identification of urinary 3-ethoxy-4-hydroxybenzoic and 3-ethoxy-4-hydroxymandelic acids after dietary intake of ethyl vanillin. *Biomed. Mass Spectrom.* 12(4):163-169.

5. Hawkins, D.R.; Midgely, I.; Proctor, P.; Parikh, R.; Bennett, S.; Huckstep, M.R. and Cheng, K. (1992) *Biokinetics and metabolism of ethyl ^{14}C-vanillin following oral (gavage) administration to rats*. Vol. 35. p.141-151. (as cited in World Health Organization, 1996).

6. World Health Organization (1996) Ethyl Vanillin. WHO Food Additives Series. p.141-151.

7. Jenner, P.M.; Hagan, E.C.; Taylor, J.M.; Cook, E.L. and Fitzhugh, O.G. (1964) Food flavourings and compounds of related structure. I. Acute oral toxicity. *Food Cosmet. Toxicol.* 2:327.

8. Deichmann, W. and Kitzmiller, K.V. (1940) On the toxicity of vanillin and ethyl vanillin for rabbits and rats. *Journal of the American Pharmaceutical Association* 29(10):425-428.

9. Grubbs, C.J.; Casebolt, T.L.; Eto, I.; Juliana, M.M.; Whitaker, L.M.; Canfield, G.J.; Steele, V.E. and Kelloff, G.J. (1994) Efficacy of ethyl vanillin in the prevention of mammary tumors induced by methylnitrosourea in the rats. *Advances in Experimental Medicine and Biology* 354(235):

10. Hagan, E.C.; Hansen, W.H.; Fitzhugh, O.G.; Jenner, P.M.; Jones, W.I.; Taylor, J.M.; Long, E.L.; Nelson, A.A. and Brouwer, J.B. (1967) Food flavourings and compounds of related structure. II. Subacute and chronic toxicity. *Food Cosmet Toxicol* 5(2):141-157.

11. Imanishi, H.; Sasaki, Y.F.; Matsumoto, K.; Watanabe, M.; Ohta, T.; Shirasu, Y. and Tutikawa, K. (1990) Suppression of 6-TG-resistant mutations in V79 cells and recessive spot formations in mice by vanillin. *Mutat Res* 243(2):151-158.

12. Morita, Y. and Mizutani, M. (1988) Effects of antimutagens on the teratogenicity of N-methyl-N'-nitro-N-nitrosoguanidine in mice. *Congen. Anom. (Senten Ijo)* 28:157-167.

13. Ishidate, M., Jr.; Sofuni, T.; Yoshikawa, K.; Hayashi, M.; Nohmi, T.; Sawada, M. and Matsuoka, A. (1984) Primary mutagenicity screening of food additives currently used in Japan. *Food Chem Toxicol* 22(8):623-636.

14. Ishidate, M.Jr. (1982) Mammalian cell systems as a screening tool for the detection of possible mutagens and/or carcinogens in the environment. *Eisei Kagaku (J Hyg Chem)* 28:291-304.

15. Jansson, T.; Curvall, M.; Hedin, A. and Enzell, C.R. (1988) In vitro studies of the biological effects of cigarette smoke condensate. III. Induction of SCE by some phenolic and related constituents derived from cigarette smoke. A study of structure-activity relationships. *Mutat Res* 206(1):17-24.

16. Wild, D.; King, M.T.; Gocke, E. and Eckhardt, K. (1983) Study of artificial flavouring substances for mutagenicity in the Salmonella/microsome, Basc and micronucleus tests. *Food Chem Toxicol* 21(6):707-719.

17. Mortelmans, K.; Haworth, S.; Lawlor, T.; Speck, W.; Tainer, B. and Zeiger, E. (1986) Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ Mutagen* 8 Suppl 7:1-119.

18. Steele, V.E.; Moon, R.C.; Lubet, R.A.; Grubbs, C.J.; Reddy, B.S.; Wargovich, M.; McCormick, D.L.; Pereira, M.A.; Crowell, J.A.; Bagheri, D. and . (1994) Preclinical efficacy evaluation of potential chemopreventive agents in animal carcinogenesis models: methods and results from the NCI Chemoprevention Drug Development Program. *J Cell Biochem Suppl* 20:32-54.

19. Furukawa, A. and Ohuchida, A. (1989) Mutagenicity test of polyploid inducers. *Mutat Res* 252(1):86-87.

20. Furukawa, A.; Ohuchida, A. and Wierzba, K. (1989) In vivo mutagenicity tests on polyploid inducers. *Environ Mol Mutagen.* 14(Suppl 15):63-64.

21. PM USA (2001) P&T/GC/MS Analysis of Ethyl Vanillin.Request 20010130. Scan TB161RFD.D. Unpublished Internal Report.

22. PM USA (2001) Pyrolysis GC/MS Analysis of Sample 01.BQ.169. Request 20010130. Scan 01B0169A.D. Unpublished Internal Report.

23. Asian Chemical Works (site visited on October 6, 2005) Gueithol. www.asianchemicals.com/pro_thr_brs_brd_gue.htm.

24. Norfoods (site visited on October 6, 2005) Ethyl Vanillin. www.norfoods.se/lundberg/eng_index.htm.

25. Carmines, E.L. (2002) Evaluation of the potential effects of ingredients added to cigarettes. Part 1: Cigarette design, testing approach, and review of results. *Food and Chemical Toxicology* 40:77-91.

26. Roemer, E.; Tewes, F.J.; Meisgen, T.J.; Veltel, D. and Carmines, E.L. (2002) Evaluation of the potential effects of ingredients added to cigarettes. Part 3: *In vitro* genotoxicity and cytotoxicity. *Food and Chemical Toxicology* 40:105-111.

27. Rustemeier, K.; Stabbert, R.; Haussmann, H.J.; Roemer, E. and Carmines E.L. (2002) Evaluation of the potential effects of ingredients added to cigarettes. Part 2: Chemical composition of mainstream smoke. *Food and Chemical Toxicology* 40:93-104.

28. Vanscheeuwijck, P.M.; Teredesai, A.; Terpstra, P.M.; Verbeek, J.; Kuhl, P.; Gerstenberg, B.; Gebel, S. and Carmines E.L. (2002) Evaluation of the potential effects of ingredients added to cigarettes. Part 4: Subchronic inhalation toxicity. *Food and Chemical Toxicology* 40:113-131.

29. Gaworski, C.L.; Dozier, M.M.; Heck, J.D.; Gerhart, J.M.; Rajendran, N.; David, R.M.; Brennecke, L.H. and Morrissey, R. (1998) Toxicologic evaluation of flavor ingredients added to cigarette tobacco: 13 week inhalation exposures in rats. *Inhal. Toxicol.* 10:357-381.

30. Gaworski, C.L.; Heck, J.D.; Bennett, M.B. and Wenk, M.L. (1999) Toxicologic evaluation of flavor ingredients added to cigarette tobacco: skin painting bioassay of cigarette smoke condensate in SENCAR mice. *Toxicology* 139(1-2):1-17.

31. Doull, J.; Frawley, J.P.; George, W.J.; Loomis, T.A.; Squire, R.A. and Taylor, S.L. (1994) A safety assessment of ingredients added to tobacco in the manufacturing of cigarettes. Covington and Burling, Washington, D.C.

32. Doull, J.; Frawley, J.P.; George, W.J.; Loomis, T.A.; Squire, R.A. and Taylor, S.L. (1998) A safety assessment of ingredients added to tobacco in the manufacturing of cigarettes. Covington and Burling, Washington, D.C.

33. Baker, R.R.; Massey, E.D. and Smith, G. (2004) An overview of the effects of tobacco ingredients on smoke chemistry and toxicity. *Food and Chemical Toxicology* 42S(Supplement 1):S53-S83.