Evaluation Summary of Cinnamaldehyde for Use as a Cigarette Ingredient

Cinnamaldehyde is extensively used in the food industry as a flavor ingredient and has been recognized as generally recognized as safe (GRAS) for use in food by Food and Drug Administration (21 CFR § 182.60) and Flavor and Extract Manufacturers Association (FEMA No. 2286).[1] The Council of Europe (CoE No. 102) also approved it for food uses.[2] Cinnamaldehyde is rapidly absorbed from the gut, metabolized to polar metabolites and excreted primarily in the urine and to a lesser extent, in the feces. Animal and human studies show a majority of the cinnamaldehyde (>90%) is excreted from the body within 24 hours.[3-11] Cinnamaldehyde has a low acute oral and dermal toxicity in laboratory animals.[12-19] In a subchronic study, gavage administration of cinnamaldehyde to rats caused a slight hyperkeratosis of the squamous portion of stomach and slight hepatic cell swelling at 500 mg/kg/day, but no effects were noted at the 125 mg/kg/day. The no observed effect level (NOEL) for this study was considered to be 125 mg/kg per day.[20]

Based on the *in vitro* mutagenicity assays in bacteria, cinnamaldehyde lacks direct mutagenic or genotoxic potentials. However, in isolated mammalian cells, cinnamaldehyde produced chromosome aberrations and/or mutations, indicating genotoxic activity.[21-29] The reported *in vitro* genotoxic activity in mammalian cells did not translate into mutagenic, clastogenic, or genotoxic activity *in vivo*.[23,24,30-40] Cinnamaldehyde was shown to have some anti-mutagenic potential.[21,41-54] In one *in vivo* gavage study, increases in the frequency of micronucleated cells in rat and mouse hepatocytes and in rat forestomach cells was noted at high doses of cinnamaldehyde.[55] As micronuclei formation was dose-dependent, it appears that induction of micronuclei is a threshold phenomenon, which occurs at extremely high levels of cinnamaldehyde intake. Furthermore, as compared to dietary or dermal exposure, the gavage doses are likely to produce much greater exposures to both the forestomach and liver. The micronuclei induction in this study cannot be considered relevant to the safety of cinnamaldehyde at normal exposure levels because of the apparent threshold and the lack of activity in other *in vivo* studies.[56]

In a primary lung-tumor assay and in a hepatocarcinogenesis study, cinnamaldehyde was not carcinogenic.[45,57-65] Cinnamaldehyde did not show any reproductive or developmental effects in the absence of high dose maternal toxicity.[66]

Dermal application of cinnamaldehyde has been reported to cause irritation and sensitization in animals and humans.[67-76] A certain degree of cross-reactivity between cinnamyl alcohol and cinnamic acid in guinea pigs sensitized to cinnamaldehyde was noted. Interestingly, with regard to the sensitizing potentials of cinnamaldehyde, considerable difference between the results from testing with 1% and 2% cinnamaldehyde has been reported. It is interesting to note that in the same group of patients, 18.7% reacted to 2% cinnamaldehyde and only 3.3% to 1% cinnamaldehyde.[77]

Cinnamaldehyde is used as a tobacco flavoring materials and may be applied directly to the tobacco during cigarette manufacturing. Cinnamaldehyde is currently used at levels below 100 ppm in selected cigarette brands manufactured and/or distributed by Philip Morris USA Inc. (PM USA) or Philip Morris Products SA (PMP SA). As such, cinnamaldehyde may be subject to

pyrolysis-type reactions when smoked. Cinnamaldehyde may also be applied to the filter as a flavoring material where it would not be subjected to pyrolysis temperatures.

As suggested by purge and trap analysis conducted by PM USA, a small portion of cinnamaldehyde would be expected to distill at 100°C.[78] However, pyrolysis studies conducted by PM USA offered evidence that cinnamaldehyde would volatilize at higher temperatures, with the production of minor pyrolysis products such us styrene. The formation of small amounts of these materials is expected, since pyrolysis of organic materials may lead to formation of these compounds.[79]

Cinnamaldehyde was 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.[80-83] Three pairs of test cigarettes were produced, each containing different groups of ingredients. Cinnamaldehyde was added to two pairs at target levels of 1, 10 or 31 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 cinnamaldehyde 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 cinnamaldehyde) added to tobacco do not add significantly to the overall toxicity of cigarettes.

Currently, information is only available for tests utilizing cinnamaldehyde in a mixture of ingredients applied to cigarette tobacco. Studies are ongoing to address the use of cinnamaldehyde as a single ingredient and at higher tobacco application levels. Published studies show there is no meaningful difference in the composition or toxicity of smoke from cigarettes with added ingredients (including cinnamaldehyde) compared to the smoke from cigarettes without added ingredients.[80-85] 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. 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* 19:151-197.

2. CoE (2000). *Chemically defined flavoring substances. Council of Europe Publication.* (2000119.

3. Caldwell, J. and Nutley, B. (1986) Comparative metabolism of cinnamic acid in rats and mice and its variation with dose. *Br. J. Pharmac* (**8**8(Suppl):423.

4. Delbressine, L.P.; Klippert, P.J.; Reuvers, J.T. and Seuttler-Berlage, F. (1981) Isolation and identification of mercapturic acids of cinnamic aldehyde and cinnamyl alcohol from urine of female rats. *Arch Toxicol* 49(1):57-64.

5. Delbressine, L.P.; Klippert, P.J.; Reuvers, J.T. and Seutter-Berlage, F. (1980) Identification of two sulphur containing urinary metabolites of cinnamic aldehyde in the rat [proceedings]. *Br J Pharmacol* 68(1):165P.

6. FDA (1982) *Toxicological principles for the safety assessment of direct food additives and color additives used in foods.* (B. o. F. USFDA). Washington, D.C.

7. Richmond, H. H. (1950) United States Rubber Company, US 2529186. *Chemical Abstracts* 29791 (1951).

8. Takenaga, M.; Hirai, A.; Terano, T.; Tamura, Y.; Kitagawa, H. and Yoshida, S. (1987) In vitro effect of cinnamic aldehyde, a main component of Cinnamomi Cortex, on human platelet aggregation and arachidonic acid metabolism. *J Pharmacobiodyn.* 10(5):201-208.

9. Teuchy, H. and Van Sumere, C.F. (1971) The metabolism of (1-^{14}C) phenylalanine, (3-^{14}C) cinnamic acid, and (2-^{14}C) ferulic acid in the rat. . *Arch. Int. Physio. Biochem.* (79):589-618.

10. Weiner, H. (1980) *Aldehyde Oxidizing Enzymes. In Enzymatic basis of Detoxication.* (Edited by William Jakoby). Vol. 1. Academic Press, New York.

11. Williams, R.T. (1959) *Detoxication Mechanisms.* John Wiley & Sons, Inc, New York. p.332-335.

12. Zaitsev, A.N. and Rakhmanina, N.L. (1974) Some data on the toxic properties of phenylethyl and cinnamyl alcohols. *Vopr Pitaniya* (6):48-53.

13. Zaitsev, A.N. and Maganova, N.B. (1975) Embryotoxic effects of some aromatizers for food products. *Vopr. Pitan* (3):64-68.

14. Sporn A. (1965) Investigation of the Toxicity of Cynamic Aldehyde. *Igiena* (14):339-346.

15. Shelanski, M. and Moldovan, M. (1973) Report to RIFM by Food and Drug Research Laboratories. Feb 16, 1973. (Cited in EPA, 2000).

16. MB Research Labs (1996) Unpublished report to RIFM. (cited in EPA, 2001). Unpublished Work.

17. Jenner, P.M.; Hagan, E.C.; Taylor, J.M.; Cook, E.L. and Fitzhugh, O.G. (1964) Food flavorings and compounds of related structure. I. Acute oral toxicity. *Food Cosmet. Toxicol.* (2):327-343.

18. Harada, M. and Ozaki, Y. (1972) [Pharmacological studies on Chinese cinnamon. I. Central effects of cinnamaldehyde]. *Yakugaku Zasshi* 92(2):135-140.

19. Fritzsche, D.a.O.I. (1986) Acute Dermal Toxicity of Cinnamaldehyde in Rabbits. Unpublished Work.

20. Herbert, C.D.; Yuan, J. and Dieter, M.P. (1994) Comparison of the toxicity of cinnamaldehyde when administered by microencapsulation in feed or by corn oil gavage. *Food Chem. Toxicol.* (32):1107-1115.

21. Azizan, A. and Blevins, R.D. (1995) Mutagenicity and antimutagenicity testing of six chemicals associated with the pungent properties of specific spices as revealed by the Ames Salmonella/microsomal assay. *Arch Environ Contam Toxicol* 28(2):248-258.

22. Blazak, W.F.; Stewart, B.E.; Galperin, I.; Allen, K.L.; Rudd, C.J.; Mitchell, A.D. and Caspary, W.J. (1986) Stable dicentric chromosomes induced by chemical mutagens in L5178Y mouse lymphoma cells. *Mutat Res* 173(4):263-266.

23. Neudecker, T. (1992) The genetic toxicology of cinnamaldehyde. *Mutat Res* 277(3):173-185.

24. Neudecker, T.; Öhrlein, K.; Eder, E. and Henschler D (1983) Effect of methyl and halogen substitutions in the C position on the mutagenicity of cinnamaldehyde. *Mutat. Res.* (110):1-8.

25. Sanyal, R.; Darroudi, F.; Parzefall, W.; Nagao, M. and Knasmuller, S. (1997) Inhibition of the genotoxic effects of heterocyclic amines in human derived hepatoma cells by dietary bioantimutagens. *Mutagenesis* 12(4):297-303.

26. 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.

27. Swales, N.J. and Caldwell, J. (1991) Cytotoxicity and depletion of glutathione (GSH) by cinnamaldehyde in rat hepatocytes. *Human Experiment. Toxicol.* (10):488-489.

28. Swales, N.J. and Caldwell, J. (1992) Cytotoxicity and glutathione depletion in hepatocytes by esters of cinnamic acid. *Human Experiment. Toxicol.* (6):589-590.

29. Swales, N.J. and Caldwell, J. (1996) Studies on trans-cinnamaldehyde II: Mechanisms of cytotoxicity in rat isolated hepatocytes. *Toxicology In Vitro.* (10):37-42.

30. Borenfreund, E. and Puerner, J.A. (1985) Toxicity determined in vitro by morphological alterations and neutral red absorption. *Toxicology Letters* 24(2-3):119-124.

31. Curvall, M.; Enzell, C.R. and Pettersson, B. (1984) An evaluation of the utility of four in vitro short term tests for predicting the cytotoxicity of individual compounds derived from tobacco smoke. *Cell Biol Toxicol* 1(1):173-193.

32. Dillon, D.; Combes, R. and Zeiger, E. (1998) The effectiveness of Salmonella strains TA100, TA102 and TA104 for detecting mutagenicity of some aldehydes and peroxides. *Mutagenesis* 13(1):19-26.

33. Dillon, D.; McGregor, D.B.; Combes, R.D. and Zeiger, E. (1992) Detection of mutagenicity in *Salmonella* of some aldehydes and peroxides. *Environ. Mol. Mutagen.* (13):19-26.

34. Durksen-Hughes, P.J.; Yang, J. and Ozcan, O. (1999) p53 Induction as a genotoxic test for twenty-five chemicals undergoing *in vivo* carcinogenicity testing. *Environ. Health Perspect.* (107):805-812.

35. Fujita, H. and Sasaki, M. (1987) Mutagenicity test of food additives with *Salmonella typhirium* TA 97 and TA102. *Annals. Tokyo Metr. Res. Lab. P. H.* (38):423-430.

36. Haines, Jr.C.E. and Shankel, D.M. (1990) The effects of cinnamaldehyde and vanillin in growth assays of *Escherichia coli* and *Salmonella typhimurium*. *Environ. Mol. Mutagen* (15(Suppl. 17)):23-24.

37. Eder, E.; Henschler, D. and Neudecker, T. (1982b) Mutagenic properties of allylic and alpha, beta-unsaturated compounds: consideration of alkylating mechanisms. *Xenobiotica* 12(12):831-848.

38. Sasaki, Y. and Endo, R. (1978) Mutagencity of aldehydes in *Salmonella*. *Mutat. Res* (54):251-252.

39. Deininger, C. and Bastian, H. (1989) Genotoxic activities of cinnamaldehydes in bacterial short-term tests. . *Naunyn-Schmiedeberg's Arch. Pharmacol* (339:): pR26.

40. Ohta, T. (1993) Modification of genotoxicity by naturally occurring flavorings and their derivatives. *Crit Rev Toxicol* 23(2):127-146.

41. de Silva, H.V. and Shankel, D.M. (1985) Effects of antimutagen cinnamaldehyde on mutation frequency in selected Ames. Salmonella tester strains. *Environ Mol Mutagen* (7 (Suppl. 3)):56-57.

42. Gocke, E. (1988) Growth inhibition and antimutagenic action by cinnamaldehyde. *Mutat. Res.* 216-293.

43. Gocke, E. and Rutten, B. (1987) Is cinnamaldehyde an antimutagen? *Mutat. Res.* (182):280-281.

44. Kuroda, K. and Yoo, Y.S. (1984) Antimutagenic activity of food additives. *Mutat. Res* (130):369.

45. Kuroda, K. and Yoo, Y.S. (1985) Rec-Assay is available for screening antimutagens as well as mutagens. In: Antimutagenesis and anticarcinogenesis mechanisms. *Basic Life Sci.* (39):565.

46. Kuroda, Y. and Inoue, T. (1988) Antimutagenesis by factors affecting DNA repair in bacteria. *Mutat Res* 202:387-392.

47. Ohta, T.; Watanabe, K.; Moriya, M.; Shirasu, Y. and Kada, T. (1983a) Antimutagenic effects of cinnamaldehyde on chemical mutagenesis in Escherichia coli. *Mutat Res* 107(2):219-227.

48. Ohta, T.; Tsukamotot, R.; Imanishi, H.; Watanabe, M. and Shirasu, Y. (1985) Effects of antimutagenic compounds on the induction of the SOS response. *Mutat. Res.* (147):271.

49. Ohta, T.; Watanabe, K.; Moriya, M.; Shirasu, Y. and Kada, T. (1983b) Antimutagenic effects of coumarin and umbelliferone on mutagenesis induced by 4-nitroquinoline 1-oxide or UV irradiation in *E. coli*. *Mutat. Res.* (117):135-138.

50. Ohta, T.; Watanabe, M.; Tsukamoto, R.; Shirasu, Y. and Kada, T. (1986) Antimutagenic effects of 5 fluorouracil and 5 fluorodeoxyuridine on UV-induced mutagenesis in *Escheria-coli*. *Mutat. Res.* (173):19-24.

51. Ohta, T.; Watanabe, K.; Moriya, M.; Shirasu, Y. and Kada, T. (1983) Analysis of the antimutagenic effect of cinnamaldehyde on chemically induced mutagenesis in Escherichia coli. *Mol Gen Genet* 192(3):309-315.

52. Sasaki, Y.F.; Imanishi, H.; Ohta, T. and Shirasu, Y. (1987) Effects of antimutagenic flavourings on SCEs induced by chemical mutagens in cultured Chinese hamster cells. *Mutat Res* 189(3):313-318.

53. Shaughnessy, D.T.; Setzer, R.W. and Demarini, D.M. (2000) Antimutagenic effect of cinnamaldehyde due to inhibition of mutations at GC sites, but not at sites in Salmonella TA104. *Environ. Mol. Mutagene.* (35):54.

54. Shaughnessy, D.T.; Setzer, R.W. and Demarini, D.M. (2001) Effect of the antimutagens vanillin and cinnamaldehyde on the spontaneous mutation spectra of *Salmonella* TA104. *Environ. Mole. Mutagene.* (37):69.

55. Mirsalis, J.C.; Tyson, C.K.; Steinmetz, K.L.; Loh, E.K.; Hamilton, C.M.; Bakke, J.P. and Spalding, J.W. (1989) Measurement of unscheduled DNA synthesis and S-phase synthesis in rodent hepatocytes following in vivo treatment: testing of 24 compounds. *Environ Mol Mutagen* 14(3):155-164.

56. Devraj, H.; Niranjali, S. and Raveendran, M. (1992) Effect of food flavor cinnamaldehdye on liver microsomal cytochrome P-450 in rats. *Bull. Environ. Contam. Toxicol.* (49):306-311.

57. Ashby, A. (1996) Prediction of rodent carcinogenicity for 30 chemicals. *Environ. Health Perspect.* (104 (Suppl. 5)):1101-1104.

58. Bootman, J. (1996) Speculations on the rodent carcinogenicity of 30 chemicals currently under evaluation in rat and mouse bioassays organised by the U.S. National Toxicology Program. *Environ Mol Mutagen* 27(3):237-243.

59. Benigni, R.; Andreoli, C. and Zito, R. (1996) Prediction of rodent carcinogenicity of further 30 chemicals bioassayed by the U.S. National Toxicology Program. *Environ Health Perspect* 104 Suppl 5:1041-1044.

60. Dornish, J.M.; Pettersen, E.O. and Oftebro, R. (1989) Modifying effect of cinnamaldehyde and cinnamaldehyde derivatives on cell inactivation and cellular uptake of cis-diamminedichloroplatinum(II) in human NHIK 3025 cells. *Cancer Res* 49(14):3917-3921.

61. Dornish, J.M.; Pettersen, E.O. and Oftebro, R. (1988) Synergistic cell inactivation of human NHIK 3025 cells by cinnamaldehyde in combination with cis-diamminedichloroplatinum(II). *Cancer Res* 48(4):938-942.

62. Kasamaki, A.; Yasuhara, T. and Urasawa, S. (1986) Tumorigenicity of Chinese hamster cells transformed with flavoring agents in nude mice. *Toxicol Lett.* (31):198.

63. King, R.D. and Srinivasan, A. (1996) Prediction of rodent carcinogenicity bioassays from molecular structure using inductive logic programming. *Environ. Health Persp* (104):1031-1040.

64. Lewis, D.J. (1981) Factors affecting the distribution of tobacco smoke-induced lesions in the rodent larynx. *Toxicology Letters* 9(2):189-194.

65. Stoner, G.D.; Shimkin, M.B.; Kniazeff, A.J.; Weisburger, J.H.; Weisburger, E.K. and Gori, G.B. (1973) Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A mice. *Cancer Res.* (33):3069-3085.

66. Mantovani, A.; Stazi, A.V.; Macri, C.; Ricciardi, C.; Piccioni, A. and Badellino, E. (1989) Pre-natal (segment II) toxicity study of cinnamic aldehyde in the Sprague-Dawley rat. *Food Chem Toxicol* 27(12):781-786.

67. Elahi, E.N.; Hotchkiss, S.A.M. and Smith, C.K. (2000) Immunochemical detection of hapten-modified proteins in cinnamaldehyde-treated human skin. *Drug Metab. Rev* (32):104.

68. Elahi, E.N. and Hotchkiss, S.A.M. (2000a) Detection of cinnamic compounds in human skin following *in* vitro exposure to cinnamaldehyde and cinnamic alcohol. *Toxicology* (148):49-50.

69. Andersen, K.E.; Volund, A. and Frankild, S. (1995) The guinea pig maximization test--with a multiple dose design. *Acta Derm. Venereol.* 75(6):463-469.

70. Basketter, D.A. (1992) Skin sensitization to cinnamic alcohol: the role of skin metabolism. *Acta Derm. Venereol.* 72(4):264-265.

71. Basketter, D.A. and Kimber, I. (1997) The local lymph node assay as a qualitative predictor of skin sensitization potency. *Human and Exptl. Tox.* (16):375.

72. Kimber, I.; Hilton, J. and Weisenberger, C. (1989) The murine local lymph node assay for identification of contact allergens: a preliminary evaluation of in situ measurement of lymphocyte proliferation. *Contact Dermatitis* 21(4):215-220.

73. Dennis, K.J. and Shibamoto, T. (1990) Photochemical products of *trans*-cinnamic alcohol: possible formation of skin irritants and allergens. *J. Toxicol. - Cut. & Ocular Toxicol. :* (9):149-157.

74. Danneman, P.J.; Booman, K.A.; Dorsky, J.; Kohrman, K.A.; Rothenstein, A.S.; Sedlak, R.I.; Steltenkamp, R.J. and Thompson, G.R. (1983) Cinnamic aldehyde: a survey of consumer patch-test sensitization. *Food Chem Toxicol* 21(6):721-725.

75. de Groot, A.C. (1999) Clinical relevance of positive patch test reactions to preservatives and fragrances. *Contact Dermatitis* 41(4):224-227.

76. Kashima, R.; Okada, J. and Ikeda, Y. (1994) Lymph node cell proliferation assay in guinea pigs for the assessment of sensitizing potentials of chemical compounds. *Food Chem Toxicol* 32(9):831-836.

77. Enders, F.; Przybilla, B. and Ring, J. (1989) Patch testing with fragrance mix at 16% and 8%, and its individual constituents. *Contact Dermatitis* 20(3):237-238.

78. PM USA (2001) P&T/GC/MS Analysis of Cinnamaldehyde. Request 20010381. Scan TE101RFD.D. Unpublished Internal Report.

79. PM USA (2001) Pyrolysis GC/MS Analysis of Cinnamaldehyde. Request 20010381. Scan 01DS234A.D. Unpublished Internal Report.

80. 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.

81. 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.

82. 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.

83. 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.

84. 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.

85. 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.