Evaluation Summary of Orange Oil, Sweet for Use as a Cigarette Ingredient

Orange oil, sweet is extensively used in the food industry as a flavor ingredient. It has been recognized as GRAS (generally recognized as safe) for use in food by U.S. Food and Drug Administration (FDA, 21 CFR § 182.20) and the Flavor and Extract Manufacturers' Association (FEMA No. 2825),[1] and is approved for use by the Council of Europe (CoE No. 143).[2] Orange oil, sweet is obtained from the tree of *Citrus sinensis* (L.) Osbeck and may be referred to as orange peel oil, orange oil, or sweet orange peel oil. The oil is a clear, mobile liquid with a yellow to orange color and an odor of fresh orange.[3] The main use of orange oil, sweet in the food industry is as a flavor ingredient.[4]

Orange oil, sweet has a low acute oral and dermal toxicity in laboratory animals.[5] Over 200 constituents have been identified in orange oil, sweet, although *d*-limonene is by far the most abundant constituent (>90% w/w).[6,7] The other minor components of orange oil, sweet are not known to have any significant toxic effects and the toxic effects of orange oil, sweet are considered to be are primarily those of *d*-limonene.[6] In a subchronic study, gavage administration of *d*-limonene (5 days/week for 13 weeks) to rats, caused an increased relative liver weight at 30 and 75 mg/kg per day. The no-observed-effect-level (NOEL) for the liver was considered as 10 mg/kg per day.[8] However, in a similar subchronic study by National Toxicology Program (NTP), no compound-related effects, except nephropathy, at levels 150 and 300 mg/kg/day, were noted.[9]

In chronic studies, *d*-limonene was tested for carcinogenicity by oral gavage in mice[9] and rats.[9,10] *d*-Limonene caused male rat-specific nephrotoxicity resulting from accumulation of the male rat-specific protein α-2μ-globulin.[11] These studies show that *d*-limonene produces renal tubular tumors in male rats by a non-DNA-reactive mechanism, through α-2μ-globulin-associated response. The mechanism by which *d*-limonene increases the incidence of renal tubular tumors in male rats is not relevant to humans as the response in male rats is uniquely linked to renal perturbation involving α-2μ-globulin.[11-13]

Developmental toxicity studies in mice suggest that *d*-limonene consumption at maternal toxic levels (2363 mg/kg/day) results in skeletal abnormalities in fetuses.[14] However, at a lower dose level (591 mg/kg/day) no maternal or fetal effects were observed.[14] In another study in rabbit, *d*-limonene was reported to be non-teratogenic at doses ranging from 250 to 1000 mg/kg/day.[15]

Orange oil, sweet and *d*-limonene have been reported to have promoting effects on the development of experimental carcinogenesis.[10,16-18] Contrary to these observations, several subsequent studies have shown that both orange oil, sweet, as well as its major constituent *d*-limonene, protects against experimental carcinogenesis.[19-31] In multiple genotoxicity studies, *d*-limonene was negative.[9,32-38] Orange oil, sweet is reported to inhibit the growth of microorganisms.[39]

Dermal application of orange oil, sweet has been reported to cause moderate irritation in animals (mice[18] and rabbits[40]) and has a weak sensitizing potential in guinea pigs.[41] In humans, orange oil, sweet is probably irritating and sensitizing.[42-46] *d*-Limonene is a skin irritant in experimental

animals[47-50] and humans.[49,51] Data from more recent studies in animals have revealed air-oxidized *d*-limonene, rather than unoxidized *d*-limonene, to be the sensitizing agent.[52-55]

Currently, orange oil, sweet 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). Orange oil, sweet is applied to cigarette tobacco as an additive, flavoring, or flavoring agent, and as such, orange oil, sweet may be subjected to pyrolysis-type reactions when smoked. Orange oil, sweet may also be applied to the filter as a flavoring material where it would not be subjected to pyrolysis temperatures.

Purge and trap and pyrolysis studies were conducted by PM USA. The results of purge and trap studies, where orange oil, sweet was heated to 100 °C, suggest that orange oil, sweet would distill at low temperatures in front of the burning cone of the tobacco.[56] Additionally, pyrolysis studies conducted with orange oil, sweet at higher temperatures suggest that orange oil, sweet would not be expected to pyrolyze.[57] *d*-Limonene, the main flavoring component of orange oil, sweet, appeared to be the most prevalent material in both of these studies.

Orange oil, sweet was a part of the PM USA ingredient testing program that was designed to evaluate the potential effects of ingredients added to typical commercial blended test cigarettes on selected biological and chemical endpoints. Orange oil, sweet was added to test cigarette tobacco at target concentrations of 100, 1000, or 10,000 ppm, and did not increase the mutagenic response of Salmonella bacteria to smoke condensate preparations.[58] Similarly, at the same target concentrations, the cytotoxic response of mouse embryo cells treated with mainstream smoke condensate preparations was not altered by orange oil, sweet addition.[59] Furthermore, the addition of orange oil, sweet to cigarette tobacco was not clastogenic/aneugenic in the bone marrow of Sprague-Dawley rats, using the *in vivo* micronucleus assay.[60] There were also no clear dose dependent increases for any smoke constituents with the addition of orange oil, sweet.[61] In conclusion, the addition of orange oil, sweet at target concentrations of 100, 1000, or 10,000 ppm did not alter the mutagenic, cytotoxic, or clastogenic affects of cigarette smoke.

The results of this evaluation of orange oil, sweet, involving a review of current published information and internal studies, suggests that the addition of orange oil, sweet as a cigarette ingredient at current levels below 100 ppm does not discernibly alter the biological effects normally associated with cigarette smoke exposure.

References

1. Hall, R.L. and Oser, B.L. (1965) Recent progress in the consideration of flavoring ingredients under the food additives amendment III. *Food Technology* 19:280.

2. CoE (2000) *Chemically-Defined Flavouring Substances*. Council of Europe Publishing, F-67075 Strasbourg Cedex, France. 149-150.

3. Burdock, G.A. (2001) *Fenaroli's Handbook of Flavor Ingredients*. CRC Press, Boca Raton, FL.

4. Matthews, R.F. and Braddock, R.J. (1987) Recovery and applications of essential oils from oranges. *Food Technol. (Chicago)* 41:57-61.

5. Opdyke, D.L.J. (1974) Orange oil expressed. Monograph on fragrance raw materials. *Food Cosmet. Toxicol.* 12:733-734.

6. Burdock, G.A. (2001) Safety Assessment of Sweet Orange Oil as a Food Ingredient. Burdock Group, Vero Beach, FL. Unpublished Work.

7. BACIS (1999) Database of Volatile Compounds in Food. TNO Nutrition and Food Research, Boelens Aroma Chemical Information Service, The Netherlands. CD-ROM.

8. Webb, D.R.; Ridder, G.M. and Alden, C.L. (1989) Acute and subchronic nephrotoxicity of *d*-limonene in Fischer 344 rats. *Food and Chemical Toxicology* 27(10):639-649.

9. National Toxicology Program (1990) Toxicology and carcinogenesis studies of *d*-limonene (CAS No. 5989-27-5) in F344/N rats and B6C3F1 mice (Gavage Studies). US Department of Health and Human Services, RTP, NC. Report No. PB90-231416.

10. Dietrich, D.R. and Swenberg, J.A. (1991) NCI-Black-Reiter (NBR) male rats fail to develop renal disease following exposure to agents that induce alpha-2u-globulin (alpha 2u) nephropathy. *Fundam. Appl Toxicol* 16(4):749-762.

11. EPA (1991) Alpha-2u-globulin: Association with chemically induced renal toxicity and neoplasia in the male rat. Washington, D.C. Report No. EPA/625/3-91/019F.

12. Hard, G.C. and Whysner, J. (1994) Risk assessment of *d*-limonene: an example of male rat-specific renal tumorigens. *Crit. Rev. Toxicol.* 24:231-254.

13. Whysner, J. and Williams, G.M. (1996) d-limonene mechanistic data and risk assessment: absolute species-specific cytotoxicity, enhanced cell proliferation, and tumor promotion. *Pharmacol Ther* 71(1-2):127-136.

14. Kodama, R.; Okubo, A.; Araki, E.; Noda, K.; Ide, H. and Ikeda, T. (1977a) Studies on *d*-limonene as a gallstone solubilizer. VII. Effects on development of mouse fetuses and offsprings. *Oyo Yakuri* 13:863-873.

15. Kodama, R.; Okubo, A.; Sato, K.; Araki, E.; Noda, K.; Ide, H. and Ikeda, T. (1977b) Studies on *d*-limonene as a gallstone solubilizer. IX. Effects on development of rabbit fetuses and offspring. *Oyo Yakuri* 13:885-898.

16. JECFA (1993) 750. Limonene (WHO Food Additive Series 30). Limonene First draft prepared by Dr. K.B. Ekelman and Dr. D. Benz. U.S. Food and Drug Administration, Washington, D.C.

17. Roe, F.J. (1959) Oil of sweet orange: a possible role in carcinogenesis. *Br J Cancer* 13(1):92-93.

18. Roe, F.J. and Peirce, W.E. (1960) Tumor promotion by citrus oils: tumors of the skin and urethral orifice in mice. *J Natl Cancer Inst* 24:1389-1403.

19. Anonymous (1988) *d*-Limonene, an anticarcinogenic terpene. *Nutrition Rev.* 46:363-365.

20. Homburger, F.; Treger, A. and Boger, E. (1971) Inhibition of murine subcutaneous and intravenous benzo(rst)pentaphene. Carcinogenesis by sweet orange oils and d-limonene. *Oncology* 25(1):1-10.

21. Wattenberg, L.W. (1983) Inhibition of neoplasia by minor dietary constituents. *Cancer Res* 43(5 Suppl):2448s-2453s.

22. Wattenberg, L.W. (1990) Inhibition of carcinogenesis by minor anutrient constituents of the diet. *Proc Nutr Soc* 49(2):173-183.

23. Wattenberg, L.W.; Hanley, A.B.; Barany, G.; Sparnins, V.L.; Lam, L.K. and Fenwick, G.R. (1985) Inhibition of carcinogenesis by some minor dietary constituents. *Princess Takamatsu Symp* 16:193-203.

24. Wattenberg, L. (1995) Chalcones, myo-inositol and other novel inhibitors of pulmonary carcinogenesis. *J Cell Biochem Suppl* 22:162-168.

25. Elson, C.E.; Maltzman, T.H.; Boston, J.L.; Tanner, M.A. and Gould, M.N. (1988) Anti-carcinogenic activity of d-limonene during the initiation and promotion/progression stages of DMBA-induced rat mammary carcinogenesis. *Carcinogenesis* 9(2):331-332.

26. Zheng, G.Q.; Kenney, P.M. and Lam, L.K.T. (1992) Effects of carvone compounds on glutathione S-transferase activity in A/J mice. *Journal of Agricultural Food Chemistry* 40:751-755.

27. Maltzman, T.H.; Hurt, L.M.; Elson, C.E.; Tanner, M.A. and Gould, M.N. (1989) The prevention of nitrosomethylurea-induced mammary tumors by d-limonene and orange oil. *Carcinogenesis* 10(4):781-783.

28. Elegbede, J.A.; Elson, C.E.; Qureshi, A.; Tanner, M.A. and Gould, M.N. (1984) Inhibition of DMBA-induced mammary cancer by the monoterpene d-limonene. *Carcinogenesis* 5(5):661-664.

29. Van Duuren, B.L. and Goldschmidt, B.M. (1976) Cocarcinogenic and tumor-promoting agents in tobacco carcinogenesis. *J Natl Cancer Inst* 56(6):1237-1242.

30. McCarthy, M.F. (1998) Selenium, calcium channel blockers, and cancer risk- the Yin and Yang of apoptosis? *Medical Hypothesis* 50:423-433.

31. Elegbede, J.A.; Elson, C.E.; Qureshi, A.; Tanner, M.A. and Gould, M.N. (1984) Inhibition of DMBA-induced mammary cancer by the monoterpene d-limonene. *Proc. Amer. Assoc. Cancer Res.* 25:127A.

32. Haworth, S.; Lawlor, T.; Mortelmans, K.; Speck, W. and Zeiger, E. (1983) Salmonella mutagenicity test results for 250 chemicals. *Environ Mutagen* 5 Suppl 1:1-142.

33. Connor, T.H.; Theiss, J.C.; Hanna, H.A.; Monteith, D.K. and Matney, T.S. (1985) Genotoxicity of organic chemicals frequently found in the air of mobile homes. *Toxicol Lett* 25(1):33-40.

34. Florin, I.; Rutberg, L.; Curvall, M. and Enzell, C.R. (1980) Screening of tobacco smokes constituents for mutagenicity using the Ames' test. *Toxicology* 18:219-232.

35. Watanabe, T.; Hiratsuka, A.; Isobe, M. and Ozawa, N. (1980) Metabolism of *d*-limonene by hepatic microsomes to non-mutagenic epoxides toward *Salmonella typhimurium*. *Biochem. Pharmacol.* 29:1068-1071.

36. Heck, J.D.; Vollmuth, T.A.; Cifone, M.A.; Jagannath, D.R.; Myhr, B. and Curren, R.D. (1989) An evaluation of food flavoring ingredients in a genetic toxicity screening battery. *Toxicologist* 1:257.

37. Kuroda, K.e.al. (1989) *Seikatsu Eisei* 33:15 (Cited in BIBRA, 1998).

38. BIBRA (1995) Toxicity profile. Orange oil. British Industrial Biological Research Association (BIBRA), Carshalton, England. Report No. T593C/CAN 108284. p.482.

39. Subba, M.S.; Soumithri, T.C. and Rao, R.S. (1967) Antimicrobial action of citrus oils. *J. Food Sci.* 32:225-227.

40. Moreno, O.M. (1973) Report to RIFM, 4 September. (Cited in Opdyke, 1974).

41. Sharp, D.W. (1978) The sensitization potential of some perfume ingredients tested using a modified draize procedure. *Toxicology* 9(3):261-271.

42. Epstein, W.L. (1973) Report to RIFM, 30 October. (Cited in Opdyke, 1974).

43. Katz, A. (1946) *Spice Mill* 69:46.

44. Meneghini, C.L. (1971) *Dermatologica* 143:137. (Cited in BIBRA, 1998).

45. York, M.; Basketter, D.A.; Cuthbert, J.A. and Neilson, L. (1995) Skin irritation testing in man for hazard assessment--evaluation of four patch systems. *Hum Exp Toxicol* 14(9):729-734.

46. Horner, S.G. (1931) Dermatits from oranges and lemons. *The Lancet* :961-962.

47. Research Institute for the Development of Fragrance Material, I. (1984) Acute dermal irritation study. Ref. Nos. 307-338/8403. (Cited in JECFA, 1993).

48. Research Institute for Fragrance Materials, I. (1985) Acute dermal irritation study; RIFM 1-32. (Cited in JECFA, 1993).

49. Klecak, G.; Geleick, H. and Frey, R. (1977) Screening of fragrance materials for allergenicity in the guinea pig. I. Comparison of four testing methods. *Journal of the Society of Cosmetic Chemists* 28:53-54.

50. Gad, S.C.; Dunn, B.J.; Dobbs, D.W.; Reilly, C. and Walsh, R.D. (1986) Development and validation of an alternative dermal sensitization test: the mouse ear swelling test (MEST). *Toxicol Appl Pharmacol* 84(1):93-114.

51. Pirila, V.; Siltanen, E. and Pirila, L. (1964) On the chemical nature of the eczematogenic agent in oil of turpentine. *Dermatologica* 128:16-21.

52. Grief, N. (1967) Cutaneous safety of fragrance material as measured by the Maximization Test. *American Perfumer and Cosmetics* 82:54-57.

53. Karlberg, A.T.; Boman, A. and Melin, B. (1991) Animal experiments on the allergenicity of d-limonene--the citrus solvent. *Ann Occup Hyg* 35(4):419-426.

54. Karlberg, A.T.; Magnusson, K. and Nilsson, U. (1992) Air oxidation of *d*-limonene (the citrus solvent) creates potent allergens. *Contact Dermatitis* 26(5):332-340.

55. Karlberg, A.T.; Shao, L.P.; Nilsson, U.; Gafvert, E. and Nilsson, J.L. (1994) Hydroperoxides in oxidized d-limonene identified as potent contact allergens. *Arch Dermatol Res* 286(2):97-103.

56. PM USA (2001) P&T/GC/MS Analysis of Sweet Orange Oil. Request 20010650. Scan TH28HCAD. Unpublished Internal Report.

57. PM USA (2001) Pyrolysis GC/MS Analysis of Sweet Orange Oil. Request 20010650. Scan P010650A.D. Unpublished Internal Report.

58. IIT Research Institute Life Sciences Group (2002) *In vitro* mutagenicity of mainstream smoke condensate (MSC) from cigarettes containing target levels of 100, 1000, and 10,000 ppm sweet orange oil in *Salmonella* reverse mutation assay. *Product Integrity Evaluation 20010650*. IITRI, Chicago, IL. Unpublished Report. Report No. 8739-115-003.

59. IIT Research Institute Life Sciences Group (2003) Cytotoxicity assay (neutral red uptake) of smoke fractions from cigarettes containing target levels of 100, 1000, and 10,000 ppm sweet orange oil. *Product Integrity Evaluation 20010650*. IITRI, Chicago, IL. Unpublished Report. Report No. 8739-115-004.

60. IIT Research Institute Life Sciences Group (2003) Evaluation of the potential clastogenic activity of smoke from cigarettes containing target levels of 100, 1000, and 10,000 ppm sweet orange oil using the micronucleus assay. *Product Integrity Evaluation 20010650*. IITRI, Chicago, IL. Unpublished Report. Report No. 8739-115-005.

61. IIT Research Institute Life Sciences Group (2004) Chemical analysis of mainstream smoke condensate (MSC) from cigarettes containing target levels of 100, 1000, and 10,000 ppm sweet orange oil. *Product Integrity Evaluation 20010650*. IITRI, Chicago, IL. Unpublished Report. Report No. 8739-115-002.