Evaluation Summary of Bergamot oil for Use as a Cigarette Ingredient

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INTRODUCTION

Currently, bergamot oil (CAS # 8007-75-8) is used worldwide at levels below **100 ppm** in selected cigarette brands manufactured and/or distributed by Philip Morris International. Bergamot oil is applied directly to the tobacco as an additive, flavoring, flavoring agent, or solvent, and as such, bergamot oil may be subject to pyrolysis-type reactions when smoked. Bergamot oil may also be applied to the filter as a flavoring material where it would not be subjected to pyrolysis temperatures. This document summarises our internal studies and current published toxicology information on bergamot oil abstracted from online toxicity databases.

TOXICITY DATA ON UN-BURNED MATERIAL

Overview

Bergamot oil is used in the food industry as a flavor ingredient. It has been listed as GRAS (Generally Recognized As Safe) for use in food by the U.S. Food and Drug Administration (FDA) (21 CFR §182.20, §582.20) and the Flavor Extract Manufacturers Association (FEMA No. 2153)[1] and is approved for use by the Council of Europe (CoE No. 137).[2] Bergamot oil is produced by cold expression of the nearly ripe fruit. The oil represents a complex mixture of over 300 compounds with 5-methoxypsoralen being the most important photoreactive ingredient in bergamot oil.[3] Bergamot oil has a long history of medicinal use, especially in Calabria, Italy.[4,5] In Europe, bergamot oil continues to be prescribed for its antiseptic properties and is part of some over-the-counter drugs.[6,7]

Health hazard data

Acute toxicity studies with bergamot oil were performed using various routes of administration. The following acute LD_{50} (the dose administered which kills half the test population) have been reported: oral LD_{50} in rat > 10 g/kg;[8] intraperitoneal LD_{50} in mouse 1.5 to 2 g/kg;[4] and dermal LD_{50} in rabbit > 20 g/kg.[9] Based on these studies, bergamot oil is practically non-toxic. 5-Methoxypsoralen (5-MOP), the most phototoxic constituent of bergamot oil, showed mutagenic activity in bacterial assays and clastogenic effects in mammalian cells in culture when exposed to UV light.[10] Additionally, 5-MOP alone and 5-MOP in bergamot oil had identical effects on cell survival and the induction of reverse mutations and recombination in diploid yeast and on cell survival and the induction of reverse and forward mutations in haploid yeast. In the presence of chemical filters, there is significant protection against the induction of genetic effects from 5-MOP and bergamot oil containing 5-MOP in haploid and diploid cells.[11] Phototoxic effects from topical application of bergamot oil were documented in both animal[12,13] and human studies.[12,14-27] However, it has been shown, in the absence of UVA irradiation, bergamot oil and 5-MOP are virtually devoid of toxicity.[16]

Bergamot oil showed sedative, anticonvulsant and analgesic effects in animals. Cardiovascular testing in animals with the non-volatile total residue of bergamot oil showed significant dilatory action and reduced hyperkinetic ventricular arrhythmias caused by post-ischemic reperfusion.[28]

TOXICITY DATA ON BURNT MATERIAL

Combustion studies

As suggested by the purge and trap studies conducted by PM USA, bergamot oil applied to tobacco would be expected to significantly distill intact at 100°C.[29] At the higher temperatures used in pyrolysis studies conducted by PM USA, results suggest that bergamot oil would not be pyrolyzed extensively and would be delivered to the smoke intact. Methoxysalen (5-methoxypsoralen) was identified as a very minor component of the pyrolysis of bergamot oil. This material is a natural component of bergamot oil, and when in the presence of UVA radiation, is considered to be a human carcinogen.[30] 5-Methoxypsoralen was not found in this sample of bergamot oil.[31]

Philip Morris ingredient testing program

Bergamot oil 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[32-35]. Three pairs of test cigarettes were produced, each containing different groups of ingredients. Bergamot oil was added to one pair at target levels of 6 ppm and 18 ppm on tobacco. 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 bergamot oil 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 bergamot oil) added to tobacco do not add significantly to the overall toxicity of cigarettes.

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.

Currently, information is only available for tests utilizing bergamot oil up to target levels of 18 ppm. Studies are ongoing to address the use of bergamot oil 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 bergamot oil) compared to the smoke from cigarettes without added ingredients.[32-37] It is our scientific judgement, based on the best available data, that bergamot oil used in our cigarettes does 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* 19:157-197.

2. Council of Europe (2000) *Natural sources of flavourings*. Koelblin-Dortuna-Druck, Strasbourg, France. p.131-132.

3. WHO (1986) 5-Methoxypsoralen. In *IARC Monographs on the Evaluation of the carcinogenic Risk of Chemicals to Humans. Some Naturally Occurring and Synthetic Food Components, Furocoumarins and Ultraviolet Radiation*. Vol. 40. IARC, Lyon, France. p.327-347.

4. Occhiuto, F.; Limardi, F. and Circosta, C. (1995) Effects of the non-volatile residue form the essential oil of citrus bergamia on the central nervous system. *International Journal of Pharmacognosy* 3:198-203.

5. Forlot, P. (1998) Perspectives of bergamot orange in pharmaceutics. *Essenze e derivati* 68(1):45-56.

6. Occhiuto, F. and Circosta, C. (1995) Effects of the non-volatile residue of bergamot oil on coronary circulation. *Acta Technol. Legis Med.* 6(3, XXI Congresso Int):365-371.

7. Rovesti, P. (1981) Therapeutic use of bergamot essential oil. *Riv. Ital. Essenze, Profumi, Piante Off. , Aromi, Saponi, Cosmet. , Aerosol* 63(September-October):306-309.

8. Fogleman, R.W. (1970a) Unpublished report cited in Opdyke (1979). *Report to RIFM, 1 July, 1970* :

9. Fogleman, R.W. (1970b) Unpublished report cited in Opdyke (1979). *Report to RIFM, 1 July, 1970* Report to RIFM, 26 August, 1970:

10. Ashwood-Smith, M.J.; Poulton, G.A.; Ceska, O.; Liu, M. and Furniss, E. (1983) An ultrasensitive bioassay for the detection of furocoumarins and other photosensitizing molecules. *Photochem. Photobiol.* 38(1):113-118.

11. Averbeck, D.; Averbeck, S.; Dubertret, L.; Young, A.R. and Morliere, P. (1990) Genotoxicity of bergapten and bergamot oil in saccharomyces cerevisiae. *Journal of Photochemistry and Photobiology. B, Biology* 7(2-4):209-229.

12. Marzulli, F.N. and Maibach, H.I. (1970) Perfume phototoxicity. *Food and Drug Administration* 21(September):695-715.

13. Yasui, Y. and Hirone, T. (1994) Action spectrum for bergamot- oil phototoxicity measured by sunburn cell counting. *Journal of Dermatology* 21(5):319-322.

14. Akyroyd, B. (1997) Bergamot--friend & foe. *Soap, Perfumery and Cosmetics* 70:35-38.

15. Opdyke, D.L.J. (1979) Monographs on fragrance raw materials. *Food Cosmet. Toxicol.* 11(6):1011-1081.

16. Dubertret, L.; Morliere, P.; Averbeck, D. and Young, A.R. (1990a) The photochemistry and photobiology of bergamot oil as a perfume ingredient: an overview. *J Photochem Photobiol B* 7(2-4):362-365.

17. Dubertret, L.; Serraf-Tircazes, D.; Jeanmougin, M.; Morliere, P.; Averbeck, D. and Young, A.R. (1990b) Phototoxic properties of perfumes containing bergamot oil on human skin: photoprotective effect of uva and uvb sunscreens. *J. Photochem. Photobiol. , B* 7(2-4):251-259.

18. Marzin, D. and Olivier, P. (1989) Study of the protective activity against photomutagenicity of 5-MOP on salmonella typhimurium TA 102 strain by U.V. filters in a suntan preparation. *Proc. Int. Congr. Psoralens* 1988(337):343.

19. Zaynoun, S.T. (1978) The quantitative analysis of bergapten in perfumes. *J. Soc. Cosmet. Chem.* 29(5):247-263.

20. Meyer, J.M. (1970) Accidents dus a un cosmetique de bronzage a base d'essence de bergamotte (In French). *Bull Soc Fr Dermatol* 77:882-884.

21. Forlot, P. (1978) Les psoralenes en photobiologie. Utilisation en cosmetologie. *J. Pharm. Belg.* 33(6):351-365.

22. Girard, J.; Unkovic, J.; Delahayes, J. and Lafille, C. (1979) Experimental study on bergamot oil phototoxicity. Correlations between man and guinea pig. *Dermatologica* 158(4):229-243.

23. Autier, P.; Dore, J.F.; Cesarini, J.P. and Boyle, P. (1997) Should subjects who used psoralen suntan activators be screened for melanoma? *Annals of Oncology* 8(5):435-437.

24. Kaddu, S.; Kerl, H. and Wolf, P. (2001) Accidental bullous phototoxic reactions to bergamot aromatherapy oil. *Journal of the American Academy of Dermatology* 45(3):458-461.

25. Cocks, H. and Wilson, D. (1998) Dangers of the intake of psoralens and subsequent UV exposure producing significant burns. *Burns* 24(1):82-.

26. Cutrone, M. and Beneforti, M. (1997) Photodermatitis in a bottle-fed baby due to liquid preparation for domestic sterilization. *Eur J Pediat Dermatol* 7:145-148.

27. Jones, P.A.; Lovell, W.W.; King, A.V. and Earl, L.K. (2001) In vitro testing for phototoxic potential using the epiderm 3-d reconstructed human skin model. *Toxicology Methods* 11(1):1-19.

28. Occhiuto, F. and Circosta, C. (1996) Cardiovascular properties of the non-volatile total residue from the essential oil of citrus bergamia. *Int. J. Pharmacogn.* 34(2):128-133.

29. PM USA (2002a) P&T/GC/MS Analysis of Bergamot oil. Request 20020297. Scan TC192JMI.D. Unpublished Internal Report.

30. IARC (1987) Chemistry and analysis of tobacco smoke. In *IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*. Vol. 27:Suppl.7. International Agency for Research on Cancer, Lyon.

31. PM USA (2002b) Pyrolysis GC/MS Analysis of Bergamot oil. Request 20020297. Scan P020297B.D. Unpublished Internal Report.

32. 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.

33. 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.

34. 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.

35. 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.

36. 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.

37. 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.