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 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 mice⁹ 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 (mice¹⁸ and rabbits⁴⁰) 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\textsuperscript{47-50} and humans.\textsuperscript{49,51} Data from more recent studies in animals have revealed air-oxidized \textit{d}-limonene, rather than unoxidized \textit{d}-limonene, to be the sensitizing agent.\textsuperscript{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.\textsuperscript{56} Additionally, pyrolysis studies conducted with orange oil, sweet at higher temperatures suggest that orange oil, sweet would not be expected to pyrolyze.\textsuperscript{57} \textit{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.\textsuperscript{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.\textsuperscript{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 \textit{in vivo} micronucleus assay.\textsuperscript{60} There were also no clear dose dependent increases for any smoke constituents with the addition of orange oil, sweet.\textsuperscript{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


