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Toxicologic evaluation of licorice extract as a cigarette ingredient ☆

E.L. Carmines *, R. Lemus, C.L. Gaworski

Philip Morris USA, P.O. Box 26583, Richmond, VA, 232616583, USA

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Abstract

Licorice extract (block, powder or liquid) may be applied to cigarette tobacco at levels of about 1–4% to enhance and harmonize the flavor characteristics of smoke, improve moisture holding characteristics of tobacco, and act as a surface active agent for ingredient application. Neat material pyrolysis studies, and smoke chemistry and biological activity studies (bacterial mutagenicity, cytotoxicity, micronucleus, and sub-chronic inhalation) with mainstream smoke, or mainstream smoke preparations from cigarettes containing various target levels (1.5–12%) of the licorice extracts were performed to provide data for an assessment of the use of licorice extract as a cigarette tobacco ingredient. At simulated tobacco burning temperatures up to 900 °C all forms of neat licorice extract pyrolyzed extensively, yielding small amounts of benzene, toluene, phenol and acetaldehyde with no indication that licorice extracts would transfer intact to mainstream smoke. As a single ingredient added to cigarette tobacco, block licorice extract at a target level of 12.5% increased smoke constituents including selected PAH, arsenic, lead, phenol and formaldehyde (on a TPM basis), while licorice extract powder (target level of 8% tobacco) increased select PAH, phenol and formaldehyde (on a TPM basis). Lower target application levels (including typical application levels) of block, powder or liquid licorice extract did not significantly alter the smoke chemistry profile. Biological tests indicated no relevant difference in the genotoxic or cytotoxic potential of either mainstream smoke (or smoke preparations) from cigarettes with added licorice extracts compared to control cigarettes. In sub-chronic 90-day rat inhalation studies, the mainstream smoke from cigarettes with 12.5% added block and 8% added powder licorice extract contained higher formaldehyde concentrations compared to control cigarette smoke. Female rats in the 12.5% block licorice extract exposure group displayed an increased incidence and severity of epithelial hyperplasia in the nose (level 2), with no relevant respiratory tract changes in the 8% powder licorice extract exposed rats. At the lower licorice extract application levels (1.25–5%), there was no indication of increased formaldehyde concentration in the smoke atmosphere and no relevant changes in respiratory tract tissues. Mineralcorticoid-like effects which have been associated with excess licorice ingestion were not found in any of the smoke inhalation studies. The results of these studies with various forms of licorice extract applied to cigarette tobacco suggest that

Abbreviations: 11βHSD, 11β-hydroxysteroid dehydrogenase; °C, degrees centigrade; CAS, chemical abstract service; CFR, Code of Federal Regulations; CPSC, US Consumer Product Safety Commission; CO, carbon monoxide; EC_{50}, concentration that reduces the number of viable cell by 50% compared to the vehicle control; DMSO, dimethyl sulfoxide; FTC, Federal Trade Commission; GRAS, generally recognized as safe; GSD, geometric standard deviation; HCN, hydrogen cyanide; HPLC, high performance liquid chromatography; IARC, International Agency for Research on Cancer; ISO, International Organization for Standardization; °K, degrees Kelvin; MMAD, mass median aerodynamic diameter; MS, mass spectrometer; MW, molecular weight; NBUA, *N*-nitrosodi-*n*-butylamine; NDMA, *N*-nitrosodimethylamine; NDEA, *N*-nitrosodiethylamine; NIST, National Institute of Standards and Technology; NNK, 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone; NMEA, *N*-nitrosomethylethylamine; NNN, N′-nitrosonornicotine; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NO*x*, Nitrogen Oxides; NPI, N-nitrosopiperidine; NPRA, *N*-nitrosodi-*n*-propylamine; NPY, *N*-nitrosopyrrolidine; NRU, neutral red uptake; OECD, Organization for Economic Cooperation and Development; PAH, polycyclic aromatic hydrocarbons; PCE, polychromatic erythrocytes; SCOGS, Select Committee on GRAS Substances; SD, standard deviation; SE, standard error; TPM, total particulate matter.

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* Corresponding author. Tel.: +1 804 274 6608; fax: +1 804 274 4047/3055.
E-mail address: edward.l.carmines@pmusa.com (E.L. Carmines).

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adding licorice extract to cigarette tobacco at levels of ⩽5% does not discernibly alter the smoke chemistry or biological effects normally associated with mainstream cigarette smoke.

1. Introduction

Records of licorice cultivation date back to the third century (Olukoga and Donaldson, 1998). It is used in two primary forms: root and extract. Licorice root contains about 20% of water-soluble extractives much of which (typically 3–5% of the root, but up to 12% in some varieties) is composed of glycyrrhizin, a mixture of potassium and calcium salts of glycyrrhizic acid. Sugars (glucose and sucrose) are also present (Dewick, 1997). Glycyrrhizin constitutes 10–25% of licorice extract and is considered the primary flavor constituent (Chandler, 1985; Samuelsson, 1992; Stormer et al., 1993). Licorice extract is produced by shredding and extracting the root. The extracted liquor is filtered and then either spray dried to produce a powder or concentrated to produce a solid block which generally has a stronger flavor than the powder (Vora, 1984). Licorice extract is also sold as a liquid solid extract where the extracted material is dissolved/suspended in a solvent to produce a syrup-like material.

Licorice and its derivatives are generally recognized as safe (GRAS), and are used in a variety of foods, some over-the-counter drugs and in both traditional and herbal medicines (21 CFR 184.1408, 310.528, 310.544, 582.10, and 582.20). The acute oral toxicity potential of glycyrrhizic acid and licorice extract is low. In mice and rats the oral LD_{50} is in the g/kg range (Komiyama et al., 1977; SCOGS, 1974). Short-term studies in both animals and humans have clearly defined the hypermineralocortocoidism effects of glycyrrhizin consumption (Card et al., 1953; Girerd et al., 1958; Kobuke et al., 1985; Komiyama et al., 1977; Molhuysen et al., 1950). Hypertension, hypokalemia, edema, and loss of plasma renin activity appear to be the most common clinical signs of glycyrrhizin toxicity. Consumption of glycyrrhizic acid by mice for 96 weeks did not elicit carcinogenic or chronic toxic effects (Kobuke et al., 1985).

Glycyrrhizic acid is not a teratogen (Food and Drug Research Laboratories, 1972; Itami et al., 1985), does not induce heritable chromosomal defects in rats or mice (Sheu et al., 1986), and is not likely to be toxic to the developing rodent fetus. Immunological studies have indicated that glycyrrhizic acid can induce the production of γ-interferon (Abe et al., 1987), with some speculation that licorice extract may have immunostimulatory properties (Utsunomiya et al., 1997). In vivo and in vitro tests have shown that glycyrrhizic acid is non-genotoxic (Litton Bionetics, 1972; Oak Ridge National Laboratory, 1982; Stanford Research Institute, 1977; Yamaguchi and Watanabe, 1984; Zani et al., 1993) and may have anti-genotoxic properties (Tanaka et al., 1987).

Licorice extract has been used since the 1880's as an additive in cigarette and pipe tobaccos and snuff (Tilley, 1948). Licorice extract is used in cigarettes both as a flavor and casing material (a mixture of hygroscopic agents and flavors used to facilitate tobacco processing). All three forms (block, powder and liquid) may be used in the production of cigarette tobacco, but they are not necessarily interchangeable because of their different flavor characteristics. Specifically licorice extract provides the following attributes (Vora, 1984):

- Enhances and harmonizes the smoke flavor.
- Reduces dryness in the mouth and throat.
- Improves moisture holding characteristics of tobacco, thus increasing stability and shelf life.
- Acts as a surface active agent during the spraying process of casing ingredients, thus improving the rate of absorption of flavors uniformly and evenly into tobacco.
- Minimizes rough smoke character by balancing out the overall flavor profile of the tobacco smoke.

As stated above, licorice extract is GRAS. Because licorice extract is added to tobacco and potentially burned during the smoking process, it is not possible to justify cigarette use based solely upon its approved use in foods. While there are no regulatory requirements for testing cigarette ingredients, in 1997, the tobacco industry and the United Kingdom reached a voluntary agreement on a testing approach for the approval and use of new ingredients in tobacco products (Secretary of State for Health, 1997). The approach suggested an evaluation of "potentially noxious components" (analysis of the constituents of smoke) and the use of biological studies such as genotoxicity and animal inhalation studies. Toxicology data on ingredients in the burnt and unburnt form known to the manufacturer are required to be submitted to member states of the European Union (2001), however, there are no specific study requirements or any guidelines for evaluation of the submitted data.

Previous studies have addressed various ingredients and mixtures of ingredients added to cigarettes (Baker et al., 2004a,b,c; Carmines, 2002; Gaworski et al., 1997, 1998, 1999; Heck et al., 2002; Stavanja et al., 2003). While some of these studies have indicated slight changes in the smoke chemistry of cigarettes containing