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Toxicological evaluation of smokeless tobacco: 2-year chronic toxicity and carcinogenicity feeding study in Wistar Han rats

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ABSTRACT

A comprehensive 2-year oral chronic toxicity/carcinogenicity study was conducted with smokeless tobacco using modern toxicological test methods and well-accepted standards. The study included a 1-year interim subgroup to assess toxicity at that intermediate time point. Test groups consisted of a tobacco blend (B) used in snus, and an aqueous tobacco extract of that tobacco blend (E) administered at 0.2, 2, or 5 mg nicotine/kg body weight/day via dosed feed to male and female Wistar Han rats. The dosages were selected to simulate potential exposure in humans ingesting smokeless tobacco or an aqueous extract of smokeless tobacco (the latter intended to simulate a snus extract, to enable bridging these data to snus epidemiology data). The following endpoints were evaluated: clinical observations, body weights, feed consumption (FC), ophthalmic exams, toxicokinetics, clinical pathology, gross pathology, and histopathology. During the 2-year study, clear treatment-related, dose-responsive effects included: (1) increases in plasma nicotine and cotinine (indicating that animals were appropriately exposed to levels relevant to human exposure) and (2) decreases in body weights with some alterations in FC. At the 2-year time point, two tumor types (in the highest B doses) displayed statistically significantly increased incidence trends vs. controls: (1) uterine carcinoma in females and (2) epididymal mesothelioma in males. Three tumor types displayed statistically significantly decreased incidence trends: (1) mammary gland adenomas in females, (2) skin basal cell carcinomas in females, and (3) thyroid follicular cell adenomas in males. These increases (and decreases) in tumor trends were interpreted as not being treatment-related because: (1) there were no preneoplastic or related non-neoplastic histopathological findings in the treated rats at the 1-year or 2-year time points to suggest that any of these neoplastic findings were treatment-related and (2) the tumor morphologies and incidences were generally within the expected range of historical controls for Wistar Han rats. Findings from this study indicate that chronic exposure of male and female Wistar Han rats to either a tobacco blend used in snus, or a tobacco extract of that blend does not lead to increased toxicity or carcinogenicity, based on the specified outcomes measured.

1. Introduction

No tobacco product has been shown to be safe and without risks. However, the increased risk to health associated with tobacco use proceeds along a pronounced continuum, influenced significantly by the type of tobacco product used and its associated toxicant profile (Zeller and Hatsukami, 2009). Cigarette smoking results in exposure to nicotine along with tobacco- and combustion-related toxicants, and is associated with an increased risk for developing chronic diseases. Largely due to the inhalation of combustion by-products, cigarette smoking significantly increases the risk of developing respiratory tract cancers (oropharyngeal, laryngeal, and lung), cardiovascular disease, and chronic obstructive pulmonary disease (Thun et al., 2000). In contrast, smokeless (non-combustible) tobacco products are largely devoid of the combustion-related toxicants; hence, their use results in exposure primarily to nicotine, along with other tobacco constituents found

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naturally in tobacco leaf or as a result of processing. The use of smokeless tobacco products is not associated with most smoking-related cancers or pulmonary disease; still, the risk of developing at least some tobacco-related diseases may be elevated relative to risks for never-users of tobacco (Henley et al., 2000).

The Strategic Dialogue on Tobacco Harm Reduction Group, a 26-member expert group of tobacco control researchers and policy makers, indicated that the convergence of results in the following areas of testing is likely to constitute the evidence base for determining the reduced risk potential of a tobacco product: (1) analysis of toxic constituents in tobacco products and smoke emissions, (2) preclinical cytotoxicity and genotoxicity in cell cultures and animal models for toxicity and disease, (3) biomarkers of exposure and effect in humans, and (4) actual health outcomes across various morbidity factors (Zeller and Hatsukami, 2009).

Consistent with establishing such an evidence base, the study reported here presents findings from a 2-year oral chronic toxicity/carcinogenicity study of a tobacco blend (snus) and an aqueous extract of that tobacco blend; in addition, a subgroup of rats was terminated at the end of year one to evaluate the potential for chronic lesion progression through the end of the bioassay. The precise oral rat exposure is somewhat different from expected snus use in humans (e.g., pouch retained in oral cavity). Nevertheless, feeding is relevant in that it simulates human ingestion of tobacco extract, providing chronic exposure data in a well-established experimental model by a similar administration route.

Krautter et al. (2008) reported the 90-day effects of tobacco ingestion in Sprague-Dawley rats, and previous studies (Theophilus et al., 2012) have confirmed the reproducibility of those effects (mainly body weight reductions) in Wistar Han rats, the test species and strain used in the current study. The data presented here further characterizes the chronic toxicity and carcinogenicity of the tobacco blend and tobacco extract under lifetime oral exposure.

The doses selected for the current study were based on preliminary palatability and dose-range finding 14-, 28- and 90-day studies (Theophilus et al., 2009, 2012) and were designed to span a no observable adverse effect level (NOAEL), a lowest observable adverse effect level (LOAEL), and a maximal tolerated dose (MTD). Using nicotine as a tracking compound at concentrations relevant to human exposure, findings from this study are consistent with those presented in the short-term studies and provide evidence that chronic oral ingestion of a smokeless tobacco blend or its extracts have limited toxicity and carcinogenicity potential in rats.

2. Materials and methods

2.1. Good laboratory practice and animal care standards

The study was conducted at Battelle, Columbus, OH, USA, in compliance with the Food and Drug Administration's Good Laboratory Practices (21 Code of Federal Regulations, Part 58).

Study animals were cared for according to the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and protocols were approved by Battelle's Institutional Animal Care and Use Committee. The complete animal care program was fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

2.2. Test articles, controls, and diets

The test articles used in diets were: (1) a smokeless tobacco blend, representative of a snus tobacco blend (26 mg nicotine/g tobacco) and (2) a water extract of that tobacco blend (23 mg nicotine/g tobacco extract). The tobacco extract (1 part tobacco blend: 8 parts potable water) was produced at 37.8 °C (1 h) and was filtered (final extract: 38% total soluble solids). The 37.8 °C was selected to mimic the normal oral temperature in humans. Test articles were stored frozen (≤0 °C).

Test articles were targeted to match nicotine contents because: (1) nicotine toxicity was expected to be dose limiting, (2) analytical methods exist for measuring nicotine, and (3) a principal tobacco constituent had to be used to standardize the dosage of the tobacco blend and extract. Thus, nicotine concentrations of the blend or extract were taken into account to prepare dosed-feed formulations of approximately equal nicotine concentration, and were used for monitoring feed formulations and rodent exposure concentrations. The dosages tested were: 0.2, 2, and 5 mg nicotine/kg BW/day. The concentration of test article in the feed was based on the anticipated feed consumption (FC) and body weight changes of Wistar Han rats to maintain constant dosages throughout the study.

Bulk test articles were analyzed for tobacco constituents and standard microbial endpoints to demonstrate that the test articles were stable during use and frozen storage conditions (data not shown). The NTP-2000 certified meal diet (Harlan Teklad Inc., Madison, WI) was used for feed formulations. Diet formulations containing the test articles were prepared monthly for the first three months and bi-monthly thereafter, and were stored at room temperature until use. Control and test article diet formulations were assayed for nicotine concentrations to confirm formulation accuracy.

Table 1
Experimental design of the 1-year chronic toxicity phase and 2-year carcinogenicity phase of the study.

	Target dosage of nicotine (mg/kg/day)	Number of rats				Dose group abbreviations	
		Males		Females		Males	Females
		Core[a,b]	TK	Core[a,b]	TK		
1 - Control (C)	0	20/60	10	20/60	10	CM	CF
2 - Carcinogenicity control (CC)[c]	0	0/60	–	0/60	–	CCM	CCF
3 - Tobacco blend low dose	0.2	20/60	10	20/60	10	B0.2M	B0.2F
4 - Tobacco blend intermediate dose	2	20/60	10	20/60	10	B2M	B2F
5 - Tobacco blend high dose	5	20/60	10	20/60	10	B5M	B5F
6 - Tobacco extract low dose	0.2	20/60	10	20/60	10	E0.2M	E0.2F
7 - Tobacco extract intermediate dose	2	20/60	10	20/60	10	E2M	E2F
8 - Tobacco extract high dose	5	20/60	10	20/60	10	E5M	E5F

[a] For each dose group (including C, but excluding CC), 20 rats/sex were assigned to the 1-year toxicity phase of the study and 10 rats/sex were used as toxicokinetic (TK) animals for plasma nicotine/cotinine analysis.
[b] For each dose group (including C and CC), 60 rats/sex were included in the 2-year carcinogenicity phase of the study.
[c] Carcinogenicity control (CC) was an independent control group that duplicated control (C) for the carcinogenicity phase of the study.