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Toxicological considerations on the use of propylene glycol as a humectant in cigarettes

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ABSTRACT

Propylene glycol (PG) is a humectant commonly used in cigarettes. Previous toxicological examinations of the effects on the addition of PG to tobacco used mixtures with several other flavoring agents. In the present work, toxicological comparisons were made of experimental cigarettes containing no added PG against otherwise similar cigarettes with three different amounts of PG added to the tobacco. The main toxicological comparison was a sub-chronic inhalation study with mainstream smoke in Sprague–Dawley rats (exposures of 150 mg/m^3 of total particulate matter, 6 h exposure per day, for 90 consecutive days). The target PG concentrations in the tobacco of the four cigarette types were 0, 4, 7 and 10%. Additional studies with mainstream smoke were bacterial mutagenicity (5 *Salmonella* strains, both with and without metabolic activation, particulate phase only), cytotoxicity of both particulate and gas/vapor phases (using the neutral red uptake assay), and analytical chemistry (41 analytes). The graded inclusion of PG into experimental cigarettes resulted in increases in the smoke concentrations of propylene oxide, at very low concentrations. Broadly similar responses were seen across the four cigarette types, and the responses were similar to those previously described in the scientific literature. The addition of PG to experimental cigarettes reduced concentrations of some smoke components (e.g. nicotine), but had minimal effects on the biological responses. Most of the changes produced in the 90-days of exposure were resolved in a 42-day post-inhalation period.

1. Introduction

Two approaches for assessing the toxicological consequences of addition of ingredients to cigarettes have been reported (1) studies using mixtures of ingredients in experimental cigarettes (Carmines, 2002; Rustemeier et al., 2002; Roemer et al., 2002; Vanscheeuwijck et al., 2002; Gaworski et al., 1998; Gaworski et al., 1999; Baker et al., 2004; Renne et al., 2006), and (2) studies using individual ingredients added to experimental cigarettes (Gaworski et al., 1997; Heck et al., 2002; Carmines et al., 2005; Carmines and Gaworski, 2005; Stavanja et al., 2003; Stavanja et al., 2006; Lemus et al., 2007; Stavanja et al., 2008). Although useful in evaluating non-specific ingredient questions, mixture studies have a limitation in that isolating potential effects related to any one specific ingredient would be difficult. When individual ingredients are tested, instead of mixtures, it is possible to use higher inclusion rates thus increasing the probability that the effects of the ingredient itself can be detected.

Propylene glycol ("PG", CAS number 57-55-6, a colorless odorless liquid, molecular weight 76.1, with a boiling point of 189 °C) is used in cigarettes as a humectant: it prevents tobacco from rapidly drying; both during manufacture and after the cigarettes are sold to consumers. Propylene glycol was included as one of 333 cigarette ingredients in one comprehensive testing program (Carmines, 2002; Rustemeier et al., 2002; Roemer et al., 2002; Vanscheeuwijck et al., 2002) and as one of 291 ingredients in another (Baker et al., 2004). This latter work used a PG inclusion rate of 8.33% (83,300 ppm). Propylene glycol was also included in an experiment using PG and glycerol, with a single PG inclusion rate of 2.18% (Heck et al., 2002). This work examined previous studies of transfer of PG from tobacco to smoke, noting that these rates may be 5–12%. There are reports on inhalation studies with "neat" PG as either vapor (Suber et al., 1989; Robertson et al., 1947), or aerosol (Suber et al., 1989), at aerosol concentrations up to 2.2 mg/l. In these studies the PG was not combusted, as in other reviews, (LaKind et al., 1999; Andersen, 1994).

Abbreviations: DNPH, dinitrophenylhydrazine; GVP, gas/vapor phase; NRU, Neutral red uptake assay; PG, Propylene glycol; PGH, high inclusion rate cigarette; PGL, low inclusion rate cigarette; PG0, the control cigarette, with no added PG; PGM, medium inclusion rate cigarette; PIP, post-inhalation period; PO, Propylene oxide; TPM, Total particulate matter.

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The primary aim of the present study was to examine the biological activity and analytical smoke chemistry of mainstream smoke from cigarettes containing three different amounts (low, medium, high) of added PG, and to compare those biological activities with that of otherwise similar cigarettes, containing no added PG. The secondary aim of the present study was to determine the amounts of PO that may be present in the smoke from experimental cigarettes containing different amounts of added PG. The cigarettes are identified in this work as "PG0" (the control, with no added PG), "PGL" (low inclusion rate), "PGM" (medium inclusion rate), and "PGH" (high inclusion rate). The target inclusion rates for addition of PG to the tobacco of PG0, PGL, PGM and PGH cigarettes were 0, 4, 7 and 10%, respectively, almost 5 times higher than the single inclusion rate in the study mentioned above (Heck et al., 2002). The target inclusion rates are approximately the same as those used in our previous study with another tobacco humectant glycerol (Carmines and Gaworski, 2005).

Propylene glycol is synthesized from propylene oxide ("PO", CAS number 75-56-9, C_3H_6O, a colorless liquid at room temperature), and PO is present at very low concentrations in the smoke from reference cigarettes (Diekmann et al., 2006). The 1R4F and 2R4F reference cigarettes were shown to have PO concentrations in mainstream smoke of 0.93 and 0.65 μg/cigarette, respectively. A chronic inhalation study with PO showed increased incidences of nasal tumors in exposed rats (Lynch et al., 1984), but a subsequent study only showed mammary tumors in PO-exposed rats (Kuper et al., 1988). Based largely on the latter study, the U.S. Environmental Protection Agency has classified PO as a Group B2, probable human carcinogen (U.S. Environmental Protection Agency, 1999). Unpublished work from our laboratories has shown that the amount of PO in smoke is largely dependent on the temperature to which the PG is heated, with clearly a large potential for such degradation in cigarettes where the tobacco may reach temperatures of up to 850 °C (Baker, 1999).

Studies were performed in compliance with local standards of Good Laboratory Practice. In the inhalation study, biological activity was assessed for each group by using the same concentration of total particulate matter (TPM) in the smoke presented to the rats. A target TPM concentration of 150 mg/m^3 was selected since when presented to experimental animals for 6 h/d and 7 d/w it represents a dynamic response range producing mild histopathological changes in the respiratory tract (Patskan and Reininghaus, 2003; Vanscheeuwijck et al., 2002; Terpstra et al., 2003; Carmines and Gaworski, 2005; Carmines et al., 2005; Lemus et al., 2007). The inhalation study was followed in some groups of rats by a 42-day period without any further exposures to smoke, termed the post-inhalation period, PIP. The inhalation study was supplemented by the *Salmonella* mutagenicity and neutral red uptake (NRU) cytotoxicity assays, along with analytical chemistry of mainstream tobacco smoke.

2. Materials and methods

2.1. Propylene glycol

The PG was purchased from a commercial U.S. supplier and was of food grade purity.

2.2. Cigarette construction

Studies were conducted with research cigarettes prepared with components (cellulose acetate filters, papers and adhesives) and construction processes consistent with commercial American cigarette manufacturing. Tobacco blends comprised bright (35%), burley (23%), Oriental (15%) and reconstituted tobacco sheet (27%). Cigarettes were 84 mm in length (57 mm tobacco rod, 27 mm filter) and 25 mm in circumference. The cellulose acetate filter contained 8% triacetin, with 30% ventilation. The cigarette paper was 100% flax and contained 0.6% potassium citrate. Adhesives were ethylene vinyl acetate based materials.

The University of Kentucky reference cigarette, 1R4F, was utilized in the studies as an internal reference to monitor study consistency and allow comparison of results with previous testing (data not shown).

2.3. Application and analysis of PG in tobacco

Solutions of PG in water were prepared at different concentrations so that a constant volume was applied to each batch of tobacco. Tobacco was prepared by spraying the PG solutions onto the tobacco prior to cigarette construction, with the intended final PG concentrations in the cigarettes targeted to be 0 ppm (PG0: control with no added PG), 40,000 ppm (PGL: low inclusion level), 70,000 ppm (PGM: medium inclusion level), and 100,000 ppm (PGH: high inclusion level).

Concentrations of PG in the solutions sprayed onto tobacco prior to cigarette manufacture were measured using gas chromatography (GC: thermal conductivity detector on 6 ft × 1/8 in. diameter stainless steel tubing with Porapak® PS 80/100 mesh column packing.) to ensure that the application solutions were correctly prepared. Additionally, cigarette tobacco analyses were also performed by GC (flame ionization detector on a RTX-35 column, 30 m × 0.25 mm, 1.0 mm film thickness) to measure applied levels. Measured PG concentrations in the tobacco before cigarette manufacture (single determination) were 0, 35,500, 65,000, and 94,000 ppm; after manufacture the concentrations (single determination) were 0, 28,900, 54,300, and 77,900 ppm. Analyses made after the completion of all the toxicology studies were complete (mean of two determinations) were 0, 24,300, 46,900, and 62,400 ppm, compared with the targets of 0, 40,000, 70,000 and 100,000 ppm. These differences between the measured PG concentrations in the test cigarette tobacco and the targeted test levels are considered representative of the typical manufacturing process for cigarettes, where losses in ingredient concentration occur as a consequence of large bulk manufacturing processes and aging.

2.4. Inhalation study

The potential for PG to alter cigarette tobacco smoke toxicity when administered by the inhalation route was investigated in a 90-d study using Sprague–Dawley rats, with special emphasis on the histopathology of the respiratory tract. The 90-d period has been shown to be suitable for the detection and comparison of smoke-related changes in both systemic toxicity and in histopathology of the respiratory tract (Coggins et al., 1980; Gaworski et al., 1998; Terpstra et al., 2003; Vanscheeuwijck et al., 2002; Carmines and Gaworski, 2005; Carmines et al., 2005; Renne et al., 2006; Stavanja et al., 2008).

2.4.1. Experimental design

Cigarettes containing added PG were compared with cigarettes containing no added PG, using diluted mainstream smoke presented nose-only to groups of male and female Sprague–Dawley rats, as reported previously (Haussmann et al., 1998; Vanscheeuwijck et al., 2002; Terpstra et al., 2003; Carmines and Gaworski, 2005; Carmines et al., 2005). The design was made with basic conformance to OECD guideline 413 (OECD, 1981). The rats were exposed 6 h/d for 90 consecutive days, followed by detailed necropsies. In some cases, sub-groups of rats were kept for a 42-day PIP, as described previously. As an additional comparative index, a group of male and female rats was exposed to air only