

# **Scientific Assessment of Ingredients Used in Our Brands**

## **September 2005**

**Carmines, E.L., "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(1): 77-91; 2002.**

### Abstract

A testing program was designed to evaluate the potential effects of 333 ingredients added to typical commercial blended test cigarettes on selected biological and chemical endpoints. Ingredients were incorporated into the test cigarettes as they are normally used in the manufacturing process. The studies performed included a bacterial mutagenicity screen (Ames assay), a mammalian cell cytotoxicity assay (neutral red uptake), determination of smoke chemical constituents, and a 90-day nose-only smoke inhalation study in rats. Three pairs of test cigarettes were produced, each containing one of three different groups of ingredients. In each pair, one of the cigarettes contained the normal approximate use level of the ingredients (low-level) and the other a 1.5-3 multiple of the normal use level (high-level). Analysis of the test cigarettes for selected ingredients or markers indicated that the target application rates were achieved and that the cigarettes had been manufactured as intended. Evaluation of cigarette performance indicated that the addition of the ingredients at high levels did not significantly alter the burning characteristics of the test cigarettes. Specific details of the individual studies conducted as part of an ingredient evaluation program are discussed in Parts 2-4 of this publication series (*Food and Chemical Toxicology*, 2002, 40, 93-104; *Food and Chemical Toxicology*, 2002, 40, 105-111; *Food and Chemical Toxicology*, 2002, 40, 113-131). The results of the smoke chemistry studies indicated a reduction in the majority of the smoke constituents and a few isolated instances of increases when compared to the control cigarettes. These smoke chemistry changes, while statistically significant, were not supported by any significant alteration in the biological effects of cigarette smoke normally seen with the bacterial mutagenicity assay, cytotoxicity assay or subchronic inhalation study. Based on the results of these studies, it can be concluded that these ingredients added to tobacco do not add significantly to the overall toxicity of cigarettes.

**Rustemeier, K.; Stabbert, R.; Haussmann, H.J.; Roemer, E.; Carmines, E.L.,**  
**“Evaluation of the potential effects of ingredients added to cigarettes. Part 2.**  
**Chemical composition of mainstream smoke”. *Food and Chemical Toxicology***  
**40(1): 93-104; 2002.**

#### Abstract

Cigarette mainstream smoke from blended research cigarettes with and without the addition of ingredients was analyzed for its chemical composition. In total, 333 ingredients commonly used in cigarette manufacturing were assigned to three different groups. Each group of ingredients was introduced at a low and a high level to the test cigarettes. The list of the 51 smoke constituents determined is based on those analytes suggested for analysis in a US Consumer Product Safety Commission proposal for low ignition cigarettes and cigarette smoke constituents identified by the International Agency for Research on Cancer as worthy of concern and characterized as carcinogens. An increase in the yield of total particulate matter (TPM) in the range of 13 to 28% relative to the control cigarette without ingredients was observed for all test cigarettes. This was presumably caused by the higher transfer rates of the added ingredients to the smoke compared to the transfer from the tobacco part of the filler. When the yields of individual constituents were normalized to the TPM yields, a reduction in the majority of the constituents was observed when compared to the control. For one of the ingredient groups this reduction was especially high: for phenols a maximum of 70%, for polycyclic aromatic hydrocarbons 50%, and for N-nitrosamines 45%. An increase in the amount relative to TPM was observed for a few smoke constituents: hydrogen cyanide and cadmium (one ingredient group), formaldehyde (one ingredient group), and resorcinol and lead (two ingredient groups). These results are consistent with the lack of any increased activity in the in vitro and in vivo assays in this same series of studies (*Food and Chemical Toxicology* 2002, 40, 105-111; *Food and Chemical Toxicology* 2002, 40, 113-131). An overall assessment of our data suggests that these ingredients, when added to the tobacco, do not add to the toxicity of smoke, even at the elevated levels tested in this series of studies.

**Roemer, E.; Tewes, F.J.; Meisgen, T.J.; Veltel, D.J.; Carmines, E.L., "Evaluation of the potential effects of ingredients added to cigarettes. Part 3. In vitro genotoxicity and cytotoxicity". *Food and Chemical Toxicology* 40(1): 105-111; 2002.**

Abstract

Cigarette mainstream smoke from blended cigarettes with and without the addition of ingredients was assayed for its cytotoxicity and genotoxicity. In total, 333 ingredients commonly used in cigarette manufacturing were assigned to three different groups. Each group of ingredients was added at a low and a high level to the test cigarettes. The mutagenicity of the particulate phase of the resulting cigarette smoke was assayed in the Salmonella plate incorporation (Ames) assay with tester strains TA98, TA100, TA102, TA1535 and TA1537. The cytotoxicity of the gas/vapor phase and the particulate phase was determined in the neutral red uptake assay with mouse embryo BALB/c 3T3 cells. Within the sensitivity and specificity of the test systems, the in vitro mutagenicity and cytotoxicity of the cigarette smoke were not increased by the addition of the ingredients.

**Vanscheeuwijck, P.M.; Teredesai, A.; Terpstra, P.M.; Verbeeck, J.; Kuhl, P.; Gerstenberg, B.; Gebel, S.; Carmines, E.L., "Evaluation of the potential effects of ingredients added to cigarettes. Part 4. Subchronic inhalation toxicity". *Food and Chemical Toxicology* 40(1): 113-131; 2002.**

Abstract

Mainstream smoke from blended research cigarettes with (test) and without (control) the addition of ingredients to the tobacco was assayed for inhalation toxicity. In total, 333 ingredients commonly used in cigarette manufacturing were assigned to three different groups. Each group of ingredients was introduced at a low and a high level to the test cigarettes. Male and female Sprague-Dawley rats were exposed nose-only either to fresh air (sham) or diluted mainstream smoke from the test, the control, or the Reference Cigarette 1R4F at a concentration of 150 microg total particulate matter/l for 90 days, 6h/day, 7 days/week. A 42-day post-inhalation period was included to evaluate reversibility of possible findings. There were no remarkable differences in in-life observations or gross pathology between test and control groups. An increase in activity of liver enzymes, known to be due to the high smoke dose, revealed no toxicologically relevant differences between the test and control groups. No toxicological differences were seen between the test and control groups for smoke-related hematological changes,

such as a decrease in total leukocyte count. The basic smoke-related histopathological effects, which were more pronounced in the upper respiratory tract than in the lower respiratory tract, were hyperplasia and squamous metaplasia of the respiratory epithelium, squamous metaplasia and atrophy of the olfactory epithelium, and accumulation of pigmented alveolar macrophages. There were no relevant qualitative or quantitative differences in findings in the respiratory tract of the rats exposed to the smoke from the control and test cigarettes. The data indicate that the addition of these 333 commonly used ingredients, added to cigarettes in three groups, did not increase the inhalation toxicity of the smoke, even at the exaggerated levels used.

**Carmines, E.L.; Lemus, R.; Gaworski, C.L., "Toxicologic evaluation of licorice extract as a cigarette ingredient". *Food and Chemical Toxicology* 43(9): 1303-1322; 2005.**

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

**Carmines, E.L.; Gaworski\*, C.L., "Toxicological evaluation of glycerin as a cigarette ingredient". *Food and Chemical Toxicology* 43(10): 1521-1539; 2005.**

#### Abstract

Glycerin is applied to cigarette tobacco at levels in the range of about 1-5% to improve moisture holding characteristics of tobacco and act as a surface active agent for flavor application. Neat material pyrolysis studies, smoke chemistry and biological activity studies (bacterial mutagenicity, cytotoxicity, in vivo micronucleus, and sub-chronic rodent inhalation) with mainstream smoke, or mainstream smoke preparations from cigarettes containing various target levels (5%, 10%, and 15%) of the glycerin were performed to provide data for an assessment of the use of glycerin as a cigarette tobacco ingredient. The actual levels of glycerin in the respective test cigarettes were determined to be 3.2%, 6.2% and 8.4% after cigarette production. At simulated tobacco burning temperatures up to 900 degrees C, neat glycerin did not pyrolyze extensively suggesting that glycerin would transfer intact to mainstream smoke (smoke was not analyzed for glycerin in this study). On a tar basis, nicotine in smoke was significantly decreased at 10% and 15% glycerin while water was increased at all addition levels. Addition of 10% or 15% glycerin also resulted in a statistically significant increase in acrolein (9%) and a decrease in acetaldehyde, propionaldehyde, aromatic amines, nitrogen oxides, tobacco specific nitrosamines, and phenols. Addition of 5% glycerin produced the same decrease in smoke constituents as the 10% and 15% groups but there was no concomitant increase in acrolein. Biological tests indicated no relevant differences in the genotoxic or cytotoxic potential of either mainstream smoke (or smoke preparations) from cigarettes with added glycerin compared to control cigarettes. Cigarette smoke atmosphere dilution, coupled with the lower nicotine delivery in the test cigarettes containing

glycerin resulted in a lower nicotine delivery to the glycerin cigarette smoke exposed rats of the 90-day inhalation study. Smoke atmosphere acrolein was also reduced in a concentration-related manner. Incorporation of glycerin at target levels up to 15% did not produce any adverse effects in rats exposed for 90-days. The major observation in the study was a reduced biological activity of the smoke as indicated by a reduction in the severity and/or incidence of focal macrophage accumulation in the lungs and goblet cell hyperplasia/hypertrophy in the nose (level 1), and goblet cell staining depletion in the nose (level 1). The results of these studies with glycerin applied to cigarette tobacco suggest that adding glycerin to cigarette tobacco at typical use levels does not adversely alter the smoke chemistry or biological effects normally associated with exposure to mainstream cigarette smoke.

**Roemer, E.; Hackenberg, U., "Mouse skin bioassay of smoke condensates from cigarettes containing different levels of cocoa". *Food Additives and Contaminants* 7(4): 563-569; 1990.**

Abstract

Smoke condensates derived from three cigarette types of identical blend to which 0, 1 and 3% cocoa powder was added to the filler were assayed on mice by chronic dermal application (skin painting). For each cigarette type, three condensate doses were applied: 60, 90 and 125 mg dry condensate/mouse/week. The results obtained in this study do not provide evidence that the biological activity of the condensates, as indicated by the occurrence of non-tumorous and tumorous lesions, is enhanced by the addition of cocoa.

**Carmines, E.L.; Lemus, R.; Gaworski, C.L.; Meisgen, T.; Rustemeier, K.; Van Miert, E.; Vetel, D., "Effects of the addition of licorice extract to tobacco on the chemical composition and biological activity of cigarette smoke". *The Toxicologist* 72(S-1): 387; 2003. Poster presented at the 42nd Society of Toxicology Meeting, Salt Lake City, UT, March 9-13, 2003. Poster**

**Merriman, L.; Carmines, E.L.; Gaworski, C.L.; Gerstenberg, B.; Meisgen, T.; Schramke, H.; Van Miert, E., "Effects of the addition of benzyl alcohol to tobacco on the chemical composition and biological activity of cigarette smoke". *The Toxicologist* 72(S-1): 293; 2003. Poster presented at the 42nd Society of Toxicology Meeting, Salt Lake City, UT, March 9-13, 2003. Poster**