

# Some Studies of the Effects of Additives on Cigarette Mainstream Smoke Properties. II. Casing Materials and Humectants\*

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## SUMMARY

Examination of extensive laboratory data collected during the past four decades, particularly those unpublished data generated in the 1950s and 1960s, indicates that none of the materials used as casing materials (sugars, licorice, cocoa) and humectants (glycerol, propylene glycol, other glycols) on smoking tobacco products, particularly cigarettes, imparts any significant adverse chemical or biological properties to the mainstream smoke (MSS) from cased and humectant-treated tobacco, a conclusion reached by DOULL *et al.* (1) in their assessment of available information on nearly 600 flavorant, casing material, and humectant ingredients variously used as cigarette tobacco additives in the U.S. Tobacco Industry.

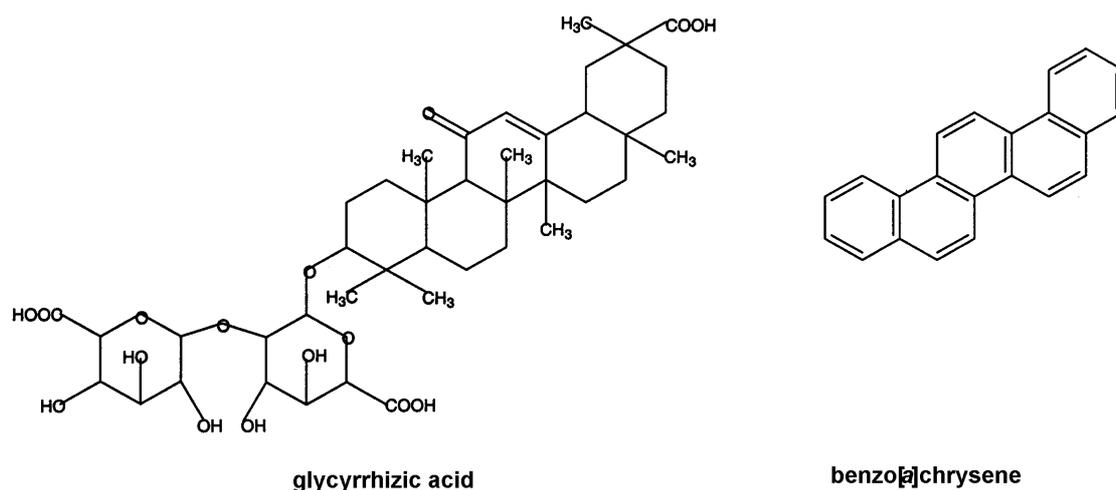
Addition of casing materials and humectants to the cigarette tobacco blend produced no significant increase in the cigarette MSS of either the total polycyclic aromatic hydrocarbon (PAH) or the benzo[*a*]pyrene (B[*a*]P) content, MSS components that have been of considerable interest for many years. Examination of the transfer of humectants from the humectant-treated tobacco to cigarette MSS indicates that the humectants act as significant diluents to the remaining MSS particulate-phase components generated from the tobacco during the smoking process. This dilution decreases the effects observed in several bioassays, e.g., mutagenicity determined in the Ames *Salmonella typhimurium* test. [Beitr. Tabakforsch. Int. 20 (2002) 279–99]

## ZUSAMMENFASSUNG

Die Überprüfung umfangreicher Untersuchungsergebnisse der letzten vier Jahrzehnte, insbesondere nicht publizierte Daten aus den fünfziger und sechziger Jahren, weisen darauf hin, dass keine der Substanzen, die als Sossierungsmittel (Zucker, Lakritze, Kakao) und Feuchthaltemittel (Glycerin, Propylenglykol, andere Glykole) zu Tabakprodukten, insbesondere Cigaretten, zugegeben wurden, signifikant nachteilige chemische oder biologische Wirkungen auf den Hauptstromrauch (HSR) dieser Cigaretten haben. Zu dieser Schlussfolgerung kamen DOULL *et al.* (1) in ihrer Beurteilung der zur Verfügung stehenden Informationen über annähernd 600 Aromatisierungs-, Sossierungs- und Feuchthaltemittel, die von der US-amerikanischen Tabakindustrie als Zusatzstoffe für Cigarettentabak benutzt wurden. Der Gesamtgehalt an polycyclischen aromatischen Kohlenwasserstoffen (PAHs) oder Benzo[*a*]pyren (B[*a*]P) im HSR, denen viele Jahre lang besonderes Interesse galt, erhöhte sich durch die Zugabe von Sossierungs- und Feuchthaltemitteln zum Cigarettentabak nicht signifikant. Die Untersuchung des Übergangs von Feuchthaltemitteln aus dem behandelten Tabak in den HSR der Cigaretten weist darauf hin, dass die Feuchthaltemittel auf die verbleibenden Partikelphasenbestandteile des HSR, die während des Rauchvorgangs produziert werden, signifikant verdünnend wirken. Diese Verdünnung vermindert die Wirkungen, die in mehreren Bioassays, z.B. Mutagenität bestimmt durch den Ames *Salmonella typhimurium* Test, beobachtet wurden. [Beitr. Tabakforsch. Int. 20 (2002) 279–99]

## RESUME

L'examen des résultats obtenus au cours des quatre dernières décennies, et surtout les données non-publiées des années 1950 et 1960, indiquent que les substances utilisées pour le sauçage (sucres, réglisse, cacao) et les humectants (glycérol, propylène glycol, autres glycols) des produits de tabacs, surtout des cigarettes, n'ont pas d'effets chimiques



**Figure 1. Glycyrrhizic acid and benz[a]chrysene**

ou biologiques significativement défavorables sur les propriétés de la fumée du courant principal (CP) des tabacs auxquels ont été ajoutés ces substances. C'est la conclusion à laquelle aboutissent DOULL *et al.* dans leur évaluation des informations disponibles sur près de 600 ingrédients apportés au tabac comme aromatisant, sauce et humectant par l'industrie du tabac aux Etats Unis.

Il a été déterminé que l'apport de sauces et d'humectants au tabac des cigarettes n'entraîne pas une augmentation significative de la teneur en hydrocarbures polycycliques aromatiques (PAH) ou en benzo[*a*]pyrene (B[*a*]P) du CP, composants du CP qui ont suscités un intérêt considérable pendant plusieurs années. L'examen du transfert des humectants du tabac traité au CP des cigarettes indique que les humectants exercent un effet de dilution significatif sur les composants de la phase particulaire du CP, générés au cours du fumage d'une cigarette. Cette dilution diminue les effets évalué par plusieurs tests biologiques, e.g. la mutagénicité déterminée au moyen du test *Salmonella typhimurium* d'Ames. [Beitr. Tabakforsch. Int. 20 (2002) 279–99]

## INTRODUCTION

Materials added to a tobacco blend during its preparation for inclusion in the final cigarette are usually classified as flavorants, casing materials, and humectants (2):

- ▶ **Flavorants:** Flavorants used on cigarette tobacco blends include menthol which may be used at a level as high as 0.8% (8 mg per gram) of the weight of the final tobacco blend and/or a variety of materials which may number as many as 40 to 100 whose total weight generally does not exceed 0.2% (2 mg per gram) of the weight of the final tobacco blend.

Occasionally, menthol is used in a “nonmenthol” cigarette at a level so low that the amount transferred to MSS is so low that its characteristic taste and odor are barely detectable to most consumers.

- ▶ **Casing materials** (3): These include sugars, licorice, and cocoa which have been used in the cigarette tobacco blend, the so-called American tobacco blend, whose first prototype was the blend of flue-cured,

burley, and Oriental tobaccos in the Camel 70-mm cigarette introduced by the R. J. Reynolds Tobacco Company (RJRT) in 1913.

It has been known for some years that a portion of the simple sugars such as glucose and fructose, either added to or inherent in the tobacco constituting the blend, is transferred to cigarette mainstream smoke (MSS) (4,5). The role of sugars in maintaining acceptability of cigarette MSS to the consumer is described by LEFFINGWELL *et al.* (3).

Because licorice and cocoa are complex mixtures, they do not transfer intact to MSS. Their compositions include compounds identical with or homologs of those identified in tobacco. Thus, many individual licorice and cocoa components behave similarly to the same or similar tobacco components during the smoking process.

Characteristic of licorice is glycyrrhizin, a potassium-calcium salt of glycyrrhizic acid (6,7) which is a polyhydric cyclohexane linked to a pentacyclic triterpenoid comprising cyclohexane rings fused in the configuration of the polycyclic aromatic hydrocarbon (PAH) benzo[*a*]chrysene (Figure 1). The structural relationship between glycyrrhizic acid, benzo[*a*]chrysene, and a phenol elicited concern in some quarters that it would generate PAHs and phenols during the smoking process (8). Licorice (3) is used to: “mellow and smooth the smoke . . . [and] . . . as an adjunct to boost the sweetness of tobacco products”. In 1981, previously published composition studies on licorice were reviewed by SCHUMACHER *et al.* (9)<sup>1</sup>.

<sup>1</sup>Numerous formal in-house reports (RDRs) and memoranda (RDMs, R&DMs, and CIMs) authored by RJRT R&D personnel are cited. Many have been published totally or in part in peer-reviewed journals and/or presented totally or in part at various scientific conferences (Tobacco Chemists' Research Conferences, American Chemical Society Symposia on Tobacco and Smoke, etc.). Whether published, presented, or neither, copies of all RJRT R&D reports cited are stored in various repositories such as the one in Minnesota. Their contents are available on the Internet at [www.rjrtdocs.com](http://www.rjrtdocs.com). The experimental procedures used, data collected, and interpretations summarized here are described in detail in the reports cited.

Characteristic of cocoa is theobromine (3,7-dimethylxanthine), a homolog of caffeine (1,3,7-trimethylxanthine), also present in cocoa but at a much lower level than theobromine. Use of cocoa as a casing material in cigarette tobacco blends was discussed by HARLLEE and LEFFINGWELL (10).

- ▶ **Humectants:** Chief among the humectants traditionally used in cigarette manufacture is glycerol and propylene glycol. Some cigarette manufacturers also use triethylene glycol.

More than 1100 materials have been proposed (11) in the scientific literature or in U.S. and foreign patents for use as tobacco additives to impart consumer-acceptable taste and/or aroma characteristics to the product and/or its smoke. Most proposed materials are highly flavorful. However, their listing (11) does not imply that all are used as cigarette ingredients. Some are utilized primarily to provide a pleasant aroma when the cigarette pack is first opened and, because of their volatility, are rapidly dissipated soon after the pack is opened. The flavorant “package” or “top dressing” is usually added to the cut tobacco blend (filler) immediately prior to cigarette fabrication (3). Many “top-dressing” components are structurally identical with or similar to identified tobacco components. With no evidence to the contrary, it is assumed that an individual added flavorant, identical with or structurally similar to a known tobacco component, would behave during the smoking process (in terms of direct transfer to smoke or degradation, reaction, etc.) much in the same manner as the naturally occurring tobacco component.

The study of tobacco additives and their contribution to smoke composition and properties is an excellent example of the significant influence of analytical methodology on our ability to generate meaningful data on the relationships between tobacco components, added components, and smoke components.

In the following, the range of addition of the various tobacco additives are presented:

Additive	Approximative addition level (mg/g tobacco blend)
<i>Casing materials</i>	
Sugars	0–25
Licorice	0–10
Cocoa	0–10
<i>Humectants</i>	
Glycerol	0–25
Propylene glycol	0–20
Triethylene glycol	0–10
<i>Flavorants</i>	
Flavor formulation <sup>a</sup>	0–2
Menthol	0–4.5

<sup>a</sup>The flavor formulation for a specific cigarette brand may consist of as many as 100 components.

The contribution to MSS composition of casing materials and humectants, because of their relatively high usage level compared to that of flavorants (excluding menthol), is much easier to study and obtain meaningful information than is the contribution of the flavorants, each of which is added to the final blend in an extremely small amount. For example, the “top dressing”, whose total weight usually

does not exceed 0.2% (2 mg/g) of the tobacco blend weight, may comprise as many as 100 individual components. In this case, the weight of each component averages about 20 micrograms per gram (20 µg/g) of tobacco filler. Sugars, licorice, and cocoa are usually classified as casing materials. Historically, these have been used as additives to pipe tobaccos and cigarette tobaccos throughout the twentieth century (3).

Humectants used on tobacco smoking products include glycerol, propylene glycol, and triethylene glycol. Glycerol has a sweet taste (12). While glycerol occurs naturally in many varieties of plants, *including tobacco* (13), propylene glycol and triethylene glycol do not. When added to tobacco, all three transfer in part during the smoking process from the tobacco to the MSS where they appear primarily in the particulate phase (14,15,16). They also appear primarily in the particulate phase of cigarette sidestream smoke (SSS) (17).

In their 1967 discussion of tobacco Additives, WYNDER and HOFFMANN (18) noted:

we need to be aware that a given additive to tobacco may have deleterious effects by increasing either the tumorigenic or toxic characteristics of the smoke. In such a case, the additive should, of course, not be used. The proof of any benefit as well as having no adverse effects needs to be established for a tobacco additive before its use can be recommended . . .

In evaluating the effect of tobacco additives, we need to consider whether such additions may contribute to the production of tumorigenic agents during the smoking of a tobacco product. If an additive increases the formation of carcinogenic substances during smoking to an analytically significant extent, it would, of course, be most undesirable. If, however, an additive should inhibit the production of tumorigenic agents during smoking and at the same time not yield other types of toxic substances, it may represent an effective and useful agent.

In terms of the “safer” or “less hazardous” cigarette, RJRT, for many years used additives – including flavorants – that were on the GRAS (Generally Regarded as Safe) and/or FEMA (Flavor and Extract Manufacturers Association) list or were known components of tobacco and/or smoke.

Ames tests with *Salmonella typhimurium* have been conducted on a variety of flavorant, casing, and humectant systems added to RJRT smoking products. Also tested has been the effect of some of these additive systems on the mutagenicity of the cigarette MSS as measured in the Ames test. These studies and the results obtained will be discussed below.

## 2 CASING MATERIALS

### 2.1 Sugars

Because all tobacco types contain several monosaccharides and disaccharides (sugars) and their levels usually range from less than 2% for burley and Maryland tobaccos to as much as 15% and more than 20% for Oriental and flue-cured tobaccos, respectively, their thermal decomposition has been studied extensively since the mid-1950s. In addition to the sugars inherent in the different tobacco types, sugars *per se* are often added as part of the casing materials formulation. Because several sugars are minor

**Table 1. Studies of possible contribution of mono-, di-, and polysaccharides to tobacco smoke**

Year	Mono- and disaccharides	Polysaccharides		
		Celluloses	Pectins	Starch
1957	GILBERT and LINDSEY (21)	GILBERT and LINDSEY (21)	GILBERT and LINDSEY (21)	GILBERT and LINDSEY (21)
1959	KOBASHI and SAKAGUCHI (4)	FREDRICKSON (22)		
1962–1963	DE LA BURDE <i>et al.</i> (24)			
1963			RODGMAN and MIMS (23)	
1965, 1966	SPEAR <i>et al.</i> (27,28)	SPEAR <i>et al.</i> (27,28)		
1965, 1967		KATO <i>et al.</i> (25,26)		
1966		WAKEHAM and SILBERMAN (113)		LATIMER (30)
1966	GARDINER (114)	GARDINER (114)		
1966–1967		SCHLOTZHAUER <i>et al.</i> (115)	SCHLOTZHAUER <i>et al.</i> (115)	
1967–1971		NEWELL and BEST (31)	NEWELL and BEST (31)	NEWELL and BEST (31)
1967		ROBB <i>et al.</i> (116)		
1970	BEST (32)			
1970	CARPENTER <i>et al.</i> (117)	HIGMAN <i>et al.</i> (54)		
1971	GAGER <i>et al.</i> (5)			
1975	PHILLPOTTS <i>et al.</i> (118)			
1975	THORNTON and MASSEY (119)			
1975	TOMITA and YOSHIDA (36)			
1976	DAVIS (120)			
1976	DICKERSON <i>et al.</i> (62)			
1976	ROBERTS <i>et al.</i> (39)	ROBERTS <i>et al.</i> (39)	ROBERTS <i>et al.</i> (39)	
1976–1981		SAKUMA <i>et al.</i> (33)		
1976, 1984		ISHIGURO <i>et al.</i> (34,35)		
1977, 1980	GORI (44), NCI (45)			
1977	DICKERSON <i>et al.</i> (78)			
1978		KUSAMA <i>et al.</i> (37)	OHNISHI <i>et al.</i> (121)	
1979		FRANKLIN (122)		
1979	SATO <i>et al.</i> (123)			
1981		OKAMOTO and YOSHIDA (38)		
1982, 1984		CARMELLA <i>et al.</i> (42)		
1983	SCHUMACHER (40)	SCHUMACHER (40)	SCHUMACHER (40)	SCHUMACHER (40)
1983		CULLIS <i>et al.</i> (124)		
1983–1986		PARK (41)		
1989		HAJALIGOL (125)		
1989		YAMAZAKI and MAEDA (126)		
1996	WEEKS (127)			
1997	COLEMAN and PERFETTI (128)	FOREHAND <i>et al.</i> (43)		

components of licorice (19,20) and cocoa, additional small quantities of several sugars are added to tobacco when these two materials are used in the casing materials formulation. The fate of sugars during smoking also led to the investigation of several of the saccharidic biopolymers in tobacco because their degradation during the smoking process was considered, after depolymerization, to approximate the degradation of the simple sugars. For example, tobacco celluloses are essentially polymers of glucose; tobacco pectins are biopolymers in which galacturonate is combined with several simple sugars (L-rhamnose, D-galactose, L-arabinose, D-xylose, L-fucose); tobacco starch is a combination of amylose and amylopectin. The simple sugars (glucose, fructose, and sucrose) and the polysaccharides (celluloses, pectins, and starch) may constitute between 40 and 50% of flue-cured tobacco. Table 1 summarizes many of the studies on the possible contribution of tobacco saccharides to smoke composition. In 1957, GILBERT and LINDSEY (21) reported that pyrolysis of simple sugars (glucose, fructose, sucrose) and other

tobacco constituents (celluloses, pectins, starch) yielded at least 17 PAHs, including B[a]P. FREDRICKSON (22) identified a series of low molecular weight aldehydes, ketones, and acids in the smoke from an all-cellulose cigarette. Subsequently, all the cellulose-derived compounds were identified in the MSS from all-tobacco cigarettes. In 1959, KOBASHI and SAKAGUCHI (4) reported the identification of several free sugars in cigarette MSS. When the study of cigarette MSS PAHs, their per cigarette deliveries, and their specific tumorigenicities in laboratory animals failed to answer several important questions in the smoking-health issue, emphasis shifted to the low molecular phenols in MSS and their promoting effect on tumorigenic PAHs. Similar to the chronological sequence with PAHs, identification of the MSS phenols was followed by research to define their precursors in tobacco. Immediately, it was realized that the major phenols precursors were different from the PAH precursors. From their 1963 study on precursors in tobacco of MSS phenols, RODGMAN and MIMS (23) reported that pectin was a major precursor of the

**Table 2. Products from pyrolysis of saccharides: their presence in tobacco smoke**

Component class	Compound identified in	
	Saccharide pyrolysate	Tobacco smoke
Hydrocarbons		
Aliphatic, saturated	5	5
Aliphatic, unsaturated	12	11
Aromatic, monocyclic	9	9
Aromatic, polycyclic	4 <sup>a</sup> (17) <sup>b</sup>	4 <sup>a</sup> (17) <sup>b</sup>
Acids	15	14
Lactones	3	3
Aldehydes	21	17
Ketones	30	22
Carbohydrates	4	4
Alcohols	3	3
Phenols	12	12
Ethers	21	8

<sup>a</sup>ROBERTS *et al.* (39) listed only 4 PAHs in saccharide pyrolysates.

<sup>b</sup>GILBERT and LINDSEY (21) listed 17 PAHs in saccharide pyrolysates.

phenols. DE LA BURDE *et al.* (24) used a radiolabeled <sup>14</sup>C-glucose to determine the fate of glucose in tobacco during a lengthy, low temperature heating sequence. 2-Furaldehyde and 5-hydroxymethyl-2-furaldehyde were identified, both known components of tobacco smoke.

In Japan, KATO *et al.* (25) and KATO (26) initiated a study of the composition of the pyrolysate from cellulose, a study that was to be continued by Japanese tobacco scientists for more than two decades (see Table 1).

In the mid-1960s, SPEARS *et al.* (27,28), using <sup>14</sup>C-glucose, reported that pyrolysis of sugars and other tobacco carbohydrates yielded phenols but noted that the tobacco carbohydrates could not account for the total phenols yield from tobacco. As noted elsewhere, the major precursors in tobacco of PAHs in smoke are the tobacco waxes (2) that include long-chained aliphatic hydrocarbons, phytosterols, and terpenoid compounds such as solanesol. The major precursors in tobacco of the simple phenols in smoke are the polymeric components of tobacco (lignin, pectins, starch, celluloses). In another pyrolysis study of glucose by JOHNSON and ALFORD (29), not only were the previously reported 2-furaldehyde and 5-hydroxymethyl-2-furaldehyde identified but also five previously unidentified compounds: 2-acetylfuran, 2-methyl-2-penten-1-one, 2-hydroxy-3-methyl-2-penten-1-one,  $\beta$ -angelica lactone, and  $\gamma$ -butyrolactone.

At RJRT R&D, an early study on pyrolysis products generated from tobacco starch was that of LATIMER (30). Eventually, this study was extended to other cell-wall constituents of tobacco. By use of radiolabeled cell-wall components from tobacco grown in a radiolabeled atmosphere, NEWELL and BEST (31) investigated the fate of the tobacco polysaccharides cellulose, pectins, and starch during the cigarette smoking process.

In 1970, BEST (32) examined the effect of sugars either inherent in or added to flue-cured tobacco on the composition and smoking quality of its smoke. From the smoking panel results it was concluded that poor smoking quality accompanied lower sugar levels.

Between 1976 and 1984, SAKUMA *et al.* (33) and ISHIGURO *et al.* (34) at the Japanese Tobacco Monopoly identified in the MSS from all-cellulose cigarettes a host of components (phenols, acids, carbonyl compounds, etc.), either previously identified or subsequently identified in tobacco smoke. ISHIGURO *et al.* (35) also reported the generation during smoldering of volatile *N*-nitrosamines from cellulose cigarettes impregnated with a variety of *N*-containing compounds (potassium nitrate, amino acids, proteins, secondary amines, nicotine) usually present in tobacco. Other Japanese scientists who investigated the behavior of cellulose during pyrolysis or smoking included TOMITA and YOSHIDA who reported on B[a]P formation (36), KUSAMA *et al.* (37), and OKAMOTO and YOSHIDA (38).

In their 1976 review of the components in pyrolysates from monosaccharides, disaccharides, and the polysaccharides cellulose and starch, ROBERTS *et al.* (39) listed over 140 pyrolysis products (see Table 2). Of these, 80% had also been identified in tobacco smoke. It should be noted that ROBERTS *et al.* listed only four PAHs identified in both the saccharide pyrolysates and tobacco smoke. In their 1957 study of tobacco saccharide pyrolysates, GILBERT and LINDSEY (21) identified 17 PAHs, all of which had been identified tobacco smoke by 1976.

In 1983, SCHUMACHER (40) reviewed not only the published data on the pyrolysis of tobacco additives and/or tobacco components but also unpublished data generated at RJRT R&D.

PARK (41) described the effect of various salts on the rate of combustion and combustion products from cellulose, particularly the cellulose in cigarette paper. In his 1987 report, PARK described the use of the yield of the cellulose pyrolysis product levoglucosan in defining the mechanism of pyrolysis.

CARMELLA *et al.* (42) investigated the effect of additives on cellulose degradation and the relationship between the pyrogenesis of catechols in MSS and the sugar, cellulose, and chlorogenic acid content of tobacco. In pyrolysis studies and cellulose cigarette MSS studies, CARMELLA *et al.* also reported that carboxymethylation of cellulose plus addition of inorganic compounds to it substantially reduced the yield of catechols.

Despite the fact that data collected for the past four decades indicate that the fate of an individual compound on pyrolysis in an inert atmosphere is not equivalent to the fate of that compound in the tobacco blend during the smoking process (2), FOREHAND *et al.* (43) attempted to demonstrate a parallelism between the pyrolysis of cellulose and the smoking of it in cigarette form.

In the National Cancer Institute (NCI) study of the third set of experimental cigarettes, the effect of invert sugars on cigarette smoke chemistry was investigated (44). The results indicated that the addition of invert sugars and glycerol did not increase the specific tumorigenicity of the cigarette smoke condensate (CSC) at the 12.5-mg/day painting dose but did increase it at the 25-mg/day dose (44,45). Addition of invert sugars alone or glycerol alone to the standard experimental blend, SEB III, produced no change in the specific tumorigenicity of the CSC. The results from the bioassays on CSCs from sugar-treated and glycerol-treated tobaccos are discussed below.

The sugars in tobacco, the enhancement of their level in the blend by addition, and their contribution to MSS composition and properties provide an interesting, perhaps confusing, array of information and/or assertions: a) Increasing the sugar level in tobacco usually lowers the "smoke pH" (46). b) High-sugar tobaccos (flue-cured, Oriental) generate CSCs with greater specific tumorigenicity than the CSCs from low-sugar tobaccos (burley, Maryland) (47). c) Increasing CSC acidity does not alter its specific tumorigenicity (48). d) Increasing the sugar level in the tobacco reduces the mutagenicity (Ames test with *Salmonella typhimurium*) of the MSS (49); glucose, fructose, galactose, sucrose, or lactose were effective with fructose producing the greatest reduction in mutagenicity. e) Sugars are precursors of phenols, alleged to be promoters of PAH tumorigenicity (50). f) Substantial reduction of level of phenols in MSS does not alter the specific tumorigenicity of the CSC (51). g) Compared to phytosterols, terpenoids, and long-chained saturated and unsaturated aliphatic hydrocarbons, sugars generate extremely low levels of PAHs, cf. (52) and (53). h) The specific tumorigenicity of the cigarette MSS from tobacco with added sugar and glycerol varies with the skin-painting dose but is not altered by inclusion of sugar alone (44,45). i) During cigarette smoking, sugars generate significant levels of low molecular weight aldehydes (formaldehyde, acetaldehyde, acrolein) (5,54). j) Consumer acceptance of cigarette MSS is proportional to the sugar content of the tobacco blend (32). k) The vapor phase of cigarette MSS is as mutagenic as the particulate phase. l) When CO and CO<sub>2</sub> are excluded, acetaldehyde is generally the most plentiful organic component in cigarette MSS vapor phase (55,56). m) Acetaldehyde is considered to be the major contributor to the mutagenicity of cigarette MSS vapor phase.

Recently, RODGMAN (57) reviewed the effect of sugars either inherent in tobacco or added to it on MSS composition and "smoke pH."

## 2.2 Licorice

Licorice, because of the polycyclic structure (a benzo[*a*]chrysene configuration) of its major specific component, glycyrrhizin (glycyrrhizic acid) (6,7), was considered by WYNDER and HOFFMANN (8) to be an additive that would be a likely source of PAHs in the smoke from tobacco to which licorice had been added. In an experiment in which the investigators attempted to compare "apples and oranges", HOFFMANN *et al.* (8) compared the level of B[*a*]P in the smoke condensate from pipe tobacco (containing 30% casing materials, including licorice [level unspecified]) smoked in a pipe to that in the smoke condensate from a cigarette tobacco similarly smoked: The pipe and cigarette tobaccos yielded 27 and 10.5 mg of B[*a*]P per 100 g of tobacco smoked, respectively. In 1966, HOFFMANN and RATHKAMP (59) reported that the pyrolysis of licorice did indeed yield PAHs (cf. GREEN and BEST [60]). The attempt to relate the results from pyrolysis studies of an individual compound or material to the results from the combustion process involving the same compound or material added to a multi-component system such as cigarette tobacco is an exercise that has many pitfalls. Such pyrolysis studies may provide qualitative information about

the nature of the products in the pyrolysate and thus provide clues to which compounds to look for in cigarette smoke. However, the yields of specific products formed by pyrolysis of an individual compound or material are always drastically different from those of the products generated when the same compound or material added to cigarette tobacco is exposed to complex series of events occurring in the smoking process.

To accumulate information on the pyrolysis products of major tobacco components as a guide to possible smoke components, RJRT R&D personnel assembled detailed bibliographies on the pyrolysis products of carbohydrates (39), amino acids and proteins (61), ammonia and sugar systems (62), and nicotine (63). The relevance of these pyrolysis topics to smoke composition is obvious. Carbohydrates (sugars, pectins, celluloses, starch), lignin, amino acids and proteins, and nicotine are the more plentiful components of tobacco and thus make significant contributions to smoke composition.

The pyrolysis of ammonia-sugar systems is important because of the sugar content of tobacco, the treatment of tobacco with ammonia as a method of denicotinization and as a means to improve its smoking quality (57,64), and the known variety of reactions between ammonia and sugars. The composition of licorice has been studied extensively, although nowhere near as extensively as that of tobacco and/or tobacco smoke. In 1983 when the identified components of tobacco and smoke numbered about 2600 and 3900, respectively (65), the identified components in licorice numbered 209. Of these 209, 172 were also known to be components of tobacco and/or tobacco smoke (9). Components identified in licorice included several of the flavorful alkylpyrazines identified in tobacco smoke. As will be seen later, structurally similar pyrazines are also flavorful components not only of the casing material cocoa (10) but also of such consumer products as peanuts, tea, coffee, roast meats, etc. (66). Also identified were several amino acids and amino acid-sugar compounds (67). The level of licorice added to tobacco may be determined by analyzing for glycyrrhizic acid (68).

In 1974, GREEN and BEST (20) examined the pyrolysis products from licorice and identified 35 components in the pyrolysate. These included phenols (phenol, 4-methoxyphenol, 2,6-dimethoxyphenol, *o*-cresol, *m*-cresol, *p*-cresol, ethylphenol, 2,6-dimethylphenol, 2-ethyl-5-methylphenol, a phenol with molecular weight = 150), 4-hydroxypyridine, and several dimethylnaphthalenes and trimethylnaphthalenes. All 35 identified components in the licorice pyrolysate are also components of tobacco smoke.

The B[*a*]P content of the licorice pyrolysate was determined and compared with that in a similarly prepared pyrolysate from flue-cured tobacco (69). The findings are summarized in Table 3.

Although it may be speculative to do so because of the various assumptions required, one may calculate from the data in Table 3 that the addition of 1% (8 mg) licorice to cigarette filler comprising flue-cured tobacco, total weight 800 mg, would make an insignificant contribution to the 15-ng B[*a*]P delivery of such a cigarette. In this calculation, it is assumed that the ratio of the B[*a*]P generated from licorice and flue-cured tobacco during the smoking process is the same as the ratio of B[*a*]P generated during the

**Table 3. Benzo[*a*]pyrene in pyrolysates from licorice and flue-cured tobacco (60)**

Material pyrolyzed	Pyrolysate (wt., mg/g pyrolyzed)	Benzo[ <i>a</i> ]pyrene	
		Total (ng)	ng/mg Pyrolysate
Licorice	117	24.8	0.21
Flue-cured tobacco	133	70.5	0.53

pyrolysis of the licorice and flue-cured tobacco. Thus, the percent contribution of the licorice to B[*a*]P in the MSS from a cigarette with 800 mg filler would be

$$100 \left( \frac{24.8 \times 8}{24.8 \times 8 + 792 \times 70.5} \right) = 0.35\%.$$

If the B[*a*]P generated in the MSS is 15 ng per cigarette, then the contribution of the licorice is  $15 \times 0.35\% = 0.053$  ng.

In 1984, VORA and TUORTO (70) reported that cigarettes treated with a small percentage of glycyrrhizic acid and smoked in a continuous or noncontinuous (puffed) burning manner yielded MSSs in both cases in which glycyrrhizic acid was found, indicating that not all of it decomposed during the smoking process. An earlier similar study was conducted on the contribution of glycyrrhizic acid and glycyrrhetinic acid added to tobacco on its smoking characteristics, particularly the taste (71).

More recently, CHUNG and ALDRIDGE (72) reported the identification of some 60 components obtained by thermal degradation of licorice as the temperature was increased from ambient to 900 °C at 20 °C/min: Compounds separated by gas chromatography (GC) and identified by MSS included 3 monocyclic aromatic hydrocarbons, 4 naphthalenes, 3 acids, 4 aldehydes, 12 ketones, 9 phenols, 2 esters, 5 ethers (all furan derivatives), and 12 *N*-containing compounds. About a dozen of the 35 components identified by GREEN and BEST (20) in the licorice pyrolysate were among the 60 identified by CHUNG and ALDRIDGE in the licorice thermogram.

### 2.3 Cocoa

Cocoa is a natural-occurring substance and is included in the casing formulation of many U.S. commercial cigarettes. Its complex composition was described by HARLLEE and LEFFINGWELL (10) in 1979. A noteworthy aspect of cocoa composition is that, in 1979, about 60% of its 252 identified volatile components were also known components of tobacco and/or tobacco smoke. Cocoa, as does licorice, contains numerous pyrazines, many of which are present in tobacco and/or tobacco smoke. As noted previously, pyrazines contribute much to the desirable taste and aroma of many foodstuffs (66). The levels of many tobacco smoke pyrazines are also significantly increased by the ammoniation of tobacco (64). Many of the identified components in cocoa contribute to its characteristic overall flavor, a flavor much enjoyed by the majority of consumers. Thus, compositionally, it appears that both licorice and cocoa are complementary to tobacco smoke. Cocoa contains theo-

bromine and a lesser amount of its homolog, caffeine. Estimation of the amount of cocoa in a tobacco blend involves analysis for theobromine (73).

Over the years, the pyrolysis of cocoa has been investigated several times. From the United States Department of Agriculture (USDA) study of the phenols generated from cocoa during pyrolysis, SCHLOTZHAUER (74) concluded that the levels of phenols from cocoa powder added to tobacco would not significantly enhance the phenol content of tobacco smoke. In their study of cocoa pyrolyzed under several different conditions in an inert atmosphere (nitrogen), namely at temperatures most likely to induce distillation, 350 and 550 °C, and to temperatures to induce pyrolysis, 650 and 850 °C, PARK *et al.* (75) reported that the major components were hydrocarbons and phenolic compounds. The pyrolysates at 350 and 550 °C were significantly different but those at 650 and 850 °C were similar. As the temperature was increased, the yields of cumene, styrene, decane, tridecane, 3-methylphenol, and 4-ethylphenol increased, yields of diacetone alcohol and hexadecane decreased. Yields of 2-methylphenol and 2-ethylphenol were not temperature dependent. The pyrolysate components identified in the study numbered 67.

Although the protein in cocoa contains about 1.5% tryptophan and 19% glutamic acid, no one has ascertained whether the pyrogenesis of the *N*-heterocyclic amines Trp-P-1, Trp-P-2, Glu-P-1, and/or Glu-P-2 from cocoa protein occurs during pyrolysis of cocoa or the smoking of cocoa-treated tobacco.

The effect on cigarette smoke composition and condensate specific tumorigenicity (mouse skin) of cocoa powder added at the 1% level to the standard experimental blend SEB III was determined in the NCI study of the third set of experimental cigarettes (44,45). It exerted a minimal effect on the phenols and PAHs deliveries, except for a 16% increase in the benz[*a*]anthracene (B[*a*]A) level in the cocoa-treated tobacco smoke condensate when compared to the condensate from SEB III with no added invert sugars, glycerol, or cocoa powder (76). These MSS chemistry data are summarized in Table 4. Discussion of the bioassay results of these and other CSCs from casing material-treated tobaccos follows.

Comparison of the smoke chemistry data for the 1% cocoa-powder-treated cigarettes with those from cigarettes containing SEB III treated with invert sugars and glycerol or with none of the three casing materials indicates very little differences in the levels of phenols and PAHs found in the smokes (76). These results are summarized in Table 4. Addition of cocoa powder (Samples Code 82 vs. 83) appeared to increase the B[*a*]A delivery by 16%. Initially, this finding caused some concern about the use of cocoa as a casing material. However, the concern lessened when was noted that in the replicate samples (Samples Code 72–75) of sugars- and glycerol-treated SEB III that the B[*a*]A delivery for the cocoa-treated sample (Sample Code 82) differs little from those of the four replicate samples. The cocoa only addition (Sample Code 82) appears to increase the B[*a*]P delivery by 6% over that of Sample Code 83. However, the B[*a*]P levels in the condensates from Samples Code 72 and 74 vary by 22% (1.16 vs. 0.95 mg/g) from the high to the low analytical values obtained. The average

**Table 4. Smoke chemistry data: NCI study on the effect of added glycerol, invert sugars, and cocoa on levels of polycyclic aromatic hydrocarbons, phenols, and aldehydes in cigarette mainstream smoke (third set of experimental cigarettes: Filler = SEB III) (44)**

Code	Additive (%) <sup>a</sup>			Microgram/g of dry condensate				No. of TBA <sup>b</sup>		MSS delivery (µg/Cigarette)			Acrolein relative to TPM (µg/mg)	
	Glycerol	Invert sugars	Cocoa	B[a]A <sup>c</sup>	B[a]P <sup>d</sup>	Phenol	m- + p-Cresol		At 12.5 mg/day	At 25.0 mg/day	Acrolein	Formaldehyde		Acetaldehyde
							o-Cresol	m- + p-Cresol						
72	2.80	5.30	0	1.43	1.16	3.86	0.78	2.09	28	50	112	34.7	1260	3.36
73	2.80	5.30	0	1.42	0.97	3.70	0.65	1.80	22	44	109	37.8	1174	3.62
74	2.80	5.30	0	1.36	0.95	3.90	0.70	1.98	30	46	105	35.3	1051	3.30
75	2.80	5.30	0	1.44	1.01	3.90	0.68	1.83	11	44	109	27.5	1205	3.46
Avg	2.80	5.30	0	1.41	1.02	3.84	0.70	1.92	23	46	109	33.8	1172	3.44
80	2.95	0	0	1.23	1.09	3.82	0.68	2.02	22	47	108	27.8	1170	3.46
81	0	5.42	0	1.50	1.08	4.22	0.72	2.07	19	41	113	32.3	1090	3.54
82	0	0	1.00	1.40	1.06	4.46	0.75	2.02	28	41	100	32.5	1067	3.32
83	0	0	0	1.21	1.00	4.33	0.68	1.98	22	30	97	33.7	1112	3.61

<sup>a</sup>GORI (129).

<sup>b</sup>TBA = tumor-bearing animals.

<sup>c</sup>B[a]A = benz[*a*]anthracene.

<sup>d</sup>B[a]P = benzo[*a*]pyrene.

B[a]P value (1.02 mg/g) for Samples Code 72 through 75 was little different from those for the SEB III treated with cocoa only (Code 82; 1.06 mg/g) and the SEB III with no casing materials at all (Code 83; 1.00 mg/g). Examination of the skin-painting bioassay data also indicates that the replicate controls (Sample Codes 72–75) gave a number of tumor-bearing animals (TBAs) ranging from 11 to 28 at the 12.5-mg/daily dosage and from 44 to 50 at the 25.0-mg/daily dosage. The TBAs for the cocoa only-treated group (Sample Code 82) fell within these limits as did the TBAs for the sugars only-treated group (Sample Code 80) and the glycerol only-treated group (Sample Code 81). Comparison of these TBA values with those for the sugars-free, glycerol-free, cocoa-free group (Sample Code 83) suggests the possibility of increased specific tumorigenicity by individual inclusion of these three casing materials. However, the TBA variations encountered in the controls (Samples Coded 72–75) raises the question: How accurate are the single TBA values obtained with Sample Group 80 at the two dosage levels? With the chemical and biological variations observed in the four replicates (Samples Code 72 through 75), it would appear to be unwise to draw sweeping conclusions based on a single analytical or biological number. A more recent study by ROEMER and HACKENBERG (77) on the effect of different cocoa levels (0, 1, and 3%) added to cigarette tobacco on the specific tumorigenicities (mouse skin-painting bioassay) of the resulting CSCs contradicted the findings reported in the NCI study of the third set of experimental cigarettes (44,45), findings which were claimed to indicate a problem with cocoa addition. Despite the fact that the 1% cocoa addition was equivalent to and the 3% cocoa addition was much greater than that used in the NCI study, ROEMER and HACKENBERG reported:

we find no evidence for indicating an enhancement of the biological activity of cigarette smoke condensates derived from cigarettes to which 1 and 3% cocoa was added.

In 1977, DICKERSON *et al.* (78) conducted a detailed study of the effect of various casing materials (invert sugars, corn syrup, licorice, cocoa) on Winston KS smoke chemistry. Each casing material was varied individually above and below its normal addition level to the Winston KS while levels of the other three were kept constant. Delivery levels of routinely monitored MSS components were determined for each variation of the four casing materials. The voluminous report on these studies contains a wealth of information on the changes in smoke chemistry produced by the variations. The highlights of the findings are the following:

- ▶ Reducing casing levels in the Winston KS decreased the MSS CO delivery and increased the MSS CO<sub>2</sub>:CO ratio.
- ▶ Invert sugars had little effect on the MSS hydrogen cyanide level.
- ▶ MSS B[a]P deliveries increased as the level of either cocoa or licorice was increased.
- ▶ The B[a]P:“tar” ratio increased but only significantly when the cocoa or licorice level was increased substantially beyond that used in the 1977 Winston KS. (The licorice level in the 1977 Winston KS was 1.2% of the blend; by 1988, the level had been reduced to 0.8%).
- ▶ The MSS level of phenol was increased as the level of added cocoa was increased. This finding paralleled that reported in the NCI study (79) of the third set of experi-

mental cigarettes even though in the NCI study only one level of cocoa addition was examined: The level of cocoa was increased from 0 to 1.00%.

- ▶ The casing materials studied by DICKERSON *et al.* showed little effect on the per cigarette MSS deliveries of acetaldehyde or acrolein, a result in agreement with data reported by GORI (79) for invert sugars or cocoa added to the standard experimental blend SEB III.

### 3 HUMECTANTS

Humectants are used in cigarettes for several purposes, including the facilitation of the cutting of tobacco (acting as a lubricant for the cutting knives) into appropriate width tobacco shreds. Their major purpose, however, is to maintain the moisture level (usually 12% at the time of cigarette manufacture) of the tobacco blend. If cigarettes lose moisture, i.e., become “dry”, during transportation, warehouse storage, shelf life, etc., their smoke composition changes drastically (80). Per cigarette MSS deliveries of “tar”, nicotine, and other smoke components (aldehydes, ketones, phenols, pyrazines) increase as the moisture level of the cigarette tobacco blend is decreased, and the smoke is perceived by the consumer as stronger, harsher, and more irritating (81).

Glycerol as a tobacco additive was studied more than a decade before the advent of the great interest in the smoking and health issue. In 1938, FORBES and HAAG (82) examined the transfer of added glycerol and diethylene glycol from the tobacco to cigarette MSS. They estimated 7 to 8% of the wet total particulate matter (WTPM) comprised glycerol and diethylene glycol. Their findings should be compared to more recent data on the humectant levels (glycerol, propylene glycol, triethylene glycol) in the Federal Trade Commission (FTC) “tar” from cigarettes marketed in the late 1970s (Table 6).

In 1949, REIF (83) described a method for the estimation of ethylene glycol in tobacco smoke. His method involved the use of 2-naphthol. Traditionally, ethylene glycol was not used in the U.S. as a tobacco humectant.

Table 5 summarizes some of the research on humectants added to tobacco. The research began in the mid-1950s and was intensified during the next decade or so. Much of the published analytical methodology and data on humectants in tobacco and cigarette MSS were provided by Tobacco Industry R&D personnel.

Because one of the degradation products of glycerol was acrolein, a 1962 report by FRANÇOIS (84) was of interest because in addition to his study of glycerol in tobacco and its smoke, he reported that no acrolein was found in the MSS from cigarettes containing tobacco impregnated with either 1.5% or 5.0% of glycerol. His finding was known to be incorrect (85).

Like many other cigarette manufacturers, RJRT traditionally has used glycerol and propylene glycol as humectants in its smoking products, i.e., cigarettes and pipe tobaccos. As noted previously, glycerol is a natural-occurring compound and was identified in the early 1960s by GREENE *et al.* (13) as a component of Oriental tobaccos (0.34–0.48%), flue-cured tobaccos (0.07–0.12%), uncased burley tobaccos (0.27–0.33%), and uncased commercial cigarette tobacco blends (0.23–0.31%) rigorously excluded from contact with glycerol or machinery exposed to glycerol. Propylene

**Table 5. Studies on humectants in tobacco and tobacco smoke: analyses, biology, fate**

Year	Glycerol	Propylene glycol	Triethylene glycol	Other
<i>Tobacco</i>				
1957	MARTIN <i>et al.</i> (130)			
1958	CUNDIFF (131)	CUNDIFF (131)		
1961	MILLER (132)	MILLER (132)	MILLER (132)	MILLER (132): 1,3-Butylene glycol Diethylene glycol
1963		CUNDIFF (133)		
1963	GREENE <i>et al.</i> (134)	GREENE <i>et al.</i> (134)		
1963	WRIGHT (135)			
1963	LANG (136)	LANG (136)	LANG (136)	LANG (136): Diethylene glycol Tetraethylene glycol
1963	PATTERSON (137)			
1965	DOIHARA <i>et al.</i> (138)	DOIHARA <i>et al.</i> (138)		
1968	SLANKI and MOSHY (139)	SLANKI and MOSHY (139)		
1969	GILES and CUNDIFF (140)	GILES and CUNDIFF (140)		
1970	GILES and GILLELAND (141)	GILES and GILLELAND (141)		
1975	DIFEE (142)	DIFEE (142)	DIFEE (142)	
1979	KUTER <i>et al.</i> (143)	KUTER <i>et al.</i> (143)		
1989	RISNER (144)	RISNER (144)		
<i>Tobacco smoke</i>				
1949				REIF (83): Ethylene glycol
1958	CARPENTER <i>et al.</i> (145)	BILL <i>et al.</i> (146)		
1959		BILL <i>et al.</i> (146)		
1960	HOLMES <i>et al.</i> (147)			
1963	GREENE <i>et al.</i> (148)	GREENE <i>et al.</i> (148)		
1963	FRIEDMAN and RAAB (149)	FRIEDMAN and RAAB (149)		
1964	LAURENE <i>et al.</i> (150)	LAURENE <i>et al.</i> (150)		
1965	LAURENE <i>et al.</i> (150)	LAURENE <i>et al.</i> (150)		
1965		LYERLY (151)		
1967		LYERLY (152)		
1974	GUERIN <i>et al.</i> (153)			
1974	SCHUMACHER <i>et al.</i> (95)	SCHUMACHER <i>et al.</i> (95)		
1975	SCHUMACHER <i>et al.</i> (154)	SCHUMACHER <i>et al.</i> (154)		
1977	SCHUMACHER <i>et al.</i> (154)	SCHUMACHER <i>et al.</i> (154)		
1986	SUMMERS (16)	SUMMERS (16)	SUMMERS (16)	
<i>Tobacco and tobacco smoke</i>				
1938	FORBES and HAAG (82)			FORBES and HAAG (82): Diethylene glycol
1962	FRANÇOIS (84)			
1965	KOBASHI <i>et al.</i> (155)	KOBASHI <i>et al.</i> (155)		
1971	CARUGNO <i>et al.</i> (156)	CARUGNO <i>et al.</i> (156)	CARUGNO <i>et al.</i> (156)	CARUGNO <i>et al.</i> (156): Diethylene glycol 1,3-Butylene glycol
1979	HEGE (15)	HEGE (15)	HEGE (15)	
1983		SWICEGOOD (96, 97)		
1999	SETTLE <i>et al.</i> (157)	SETTLE <i>et al.</i> (157)		
<i>Degradation</i>				
1962	FRANÇOIS (84)			
1964	DOIHARA <i>et al.</i> (158)	DOIHARA <i>et al.</i> (158)		
1964	KRÖLLER (159)	KRÖLLER (159)	KRÖLLER (159)	KRÖLLER (159): Diethylene glycol 1,3-Propylene glycol Sorbitol
1965	KRÖLLER (160)	KRÖLLER (160)	KRÖLLER (160)	KRÖLLER (160): Diethylene glycol 1,3-Propylene glycol Sorbitol
1999		KAGAN <i>et al.</i> (161)		

**Table 5 (contd.)**

Year	Glycerol	Propylene glycol	Triethylene glycol	Other
<i>Bioassays</i>				
1979	Bio-Research Laboratories Ltd. (104)	Bio-Research Laboratories Ltd. (104)	Bio-Research Laboratories Ltd. (104)	
1987		SUBER <i>et al.</i> (106)		
1988	GREENSPAN <i>et al.</i> (107)	GREENSPAN <i>et al.</i> (107)		
1988	LEE <i>et al.</i> (108)			
1989	DOOLITTLE and LEE (109)			
1990	FULP <i>et al.</i> (162)			
1990	MOSBERG <i>et al.</i> (163)	MOSBERG <i>et al.</i> (163)		
1999	GAWORSKI <i>et al.</i> (110)	GAWORSKI <i>et al.</i> (110)		
<i>Reviews of Humectant Studies</i>				
1964	WYNDER and HOFFMANN (164)	WYNDER and HOFFMANN (164)		
1967	WYNDER and HOFFMANN (165)	WYNDER and HOFFMANN (165)		
1991	BEST (166)	BEST (166)		

glycol is a synthetic compound not found in nature. Glycerol and propylene glycol transfer from tobacco to MSS from filtered and unfiltered cigarettes (14,15) as does triethylene glycol (15). In 1963, GREENE *et al.* (14) reported the transfer of glycerol and propylene glycol cigarette tobacco to MSS to be 5.2% and 4.5%, respectively.

It has been known for many years that oxidation of glycerol yields acrolein, a potent lachrymator. It was first identified at RJRT R&D in cigarette MSS by LAURENE *et al.* (85) in 1959. From their observations on the MSSs from all-tobacco and from all-cellulose cigarettes, LAURENE *et al.* suggested that a major precursor of acrolein in cigarette MSS was the tobacco cellulose:

Though no reliable quantitative results have been obtained for acrolein, the chromatographic peak including this compound was more than six times as large for cellulose as it normally is for cigarettes.

Because acrolein is extremely irritating to the respiratory tract, was identified as a component of the vapor phase of cigarette MSS, and was named as a potent ciliostat in the *in vitro* ciliated systems used by KENSLER and BATTISTA (86) and others, a concerted effort was mounted at RJRT (and elsewhere) to develop an appropriate analytical method for its quantitation in cigarette MSS (87,88).

In 1960, BENTLEY and BURGAN (89) of Imperial Tobacco (UK) investigated the effect of 27 compounds (6 organic compound, 21 inorganic compounds) added individually to tobacco on the B[a]P content of the MS CSC. Of the inorganic compounds studied as additives only the nitrates and a nitrite were effective in reducing the MSS B[a]P level. They reported that individual additions to flue-cured tobacco of 3% glycerol and 3% ethylene glycol gave substantial reductions, 62% and 56%, respectively, in the MSS B[a]P deliveries. Their smoking regime involved puffs of 15-mL volume, 2-sec duration, 4 Puff/min. WYNDER and HOFFMANN (90) questioned their MSS B[a]P results with glycerol- and ethylene glycol-treated tobacco as well as the B[a]P results obtained with several of the other compounds tested:

Six of the tested additives [potassium nitrate, copper (II) nitrate, sodium nitrite, glycerol, propylene glycol, ammonium

sulfate] were reportedly effective in reducing BaP . . . Some of the data presented by Bentley and Burgan appear questionable . . . ; nevertheless, the reduction of BaP by copper (II) nitrate could be qualitatively reconfirmed by Wynder and Hoffmann [91].

Later at Imperial Tobacco (Canada), DESOUZA and SCHERBAK (92), using an improved experiment design, a more reproducible analytical method for B[a]P, and the smoking regime used in most studies (35-mL puff, 2-sec puff duration, 1 puff/min) were unable to confirm the BENTLEY-BURGAN B[a]P findings. As the glycerol content of the tobacco blend was increased from 0% to 3.3% to 6.1%, DESOUZA and SCHERBAK reported that the MSS B[a]P increased from 33.5 ng/cig to 34.8 ng/cig to 35.1 ng/cig, respectively, while the nicotine delivery decreased from 2.12 mg/cig to 2.06 mg/cig to 2.02 mg/cig, respectively. However, DESOUZA and SCHERBAK considered the MSS B[a]P and nicotine deliveries to be “essentially unaltered” by the changes in the levels of added glycerol.

In the NCI “less hazardous” cigarette program, several additives were studied in the third set of standard experimental blend (SEB III) cigarettes (93). The MSS data, summarized in Table 4, indicate that addition of glycerol at a 2.95% level to SEB III produced an 11% increase in per cigarette delivery of acrolein (cf. Codes 80 vs. 83), but a slight reduction (4%) in acrolein per milligram of TPM delivered (94).

Addition of invert sugars to SEB III produced a 16% increase in per cigarette delivery of acrolein (cf. Codes 81 vs. 83, Table 4), whereas addition of both invert sugars and glycerol to SEB III produced a 12% increase in per cigarette acrolein (cf. average for Codes 72–75 vs. Code 83, Table 4).

During the separation of the highly polar components in the water-soluble portion of MS CSC, SCHUMACHER *et al.* (95) were faced with the task of separating large amounts of the humectants glycerol and propylene glycol from the remaining water-soluble components present at much lower concentrations. This eventually led to examination of the contribution of humectants to the FTC “tar” and consideration of the possibility of additional modest control of FTC

**Table 6. Humectants in cigarette mainstream smoke: their contribution to the FTC “tar” value (15)**

Brand (1979)	FTC “tar” <sup>a</sup>	Glycerol		Propylene glycol		Triethylene glycol		Total humectants		% of FTC “tar”
		Tobacco <sup>b</sup>	MSS <sup>a</sup>							
Winston KS	18.6	21.5	1.55	6.9	0.55	—	—	28.4	2.10	11.2
Winston B KS	15.8	18.6	1.35	18.8	0.70	—	—	37.4	2.05	13.0
Winston Lights KS	13.2	19.2	1.20	5.5	0.36	—	—	24.7	1.56	11.8
Camel Lights KS	10.9	17.9	1.23	3.7	0.23	—	—	21.6	1.46	13.4
Real KS	10.5	18.0	1.01	1.3	0.13	—	—	21.3	1.14	10.9
Now KS	1.7	18.0	0.26	3.6	0.04	—	—	21.6	0.30	17.4
Salem KS	17.1	18.5	1.44	7.0	0.60	—	—	25.5	2.04	11.9
Marlboro KS	15.6	18.9	1.27	11.4	0.75	7.6	1.26	37.9	3.28	21.0
Merit KS	8.2	20.3	1.01	12.5	0.42	7.1	0.71	39.9	2.14	26.1
Marlboro Lights KS	10.4	20.3	1.11	11.9	0.54	7.4	0.85	39.6	2.53	24.3
Kent Golden Lights KS	7.5	21.6	1.27	9.8	0.32	—	—	31.4	1.59	21.2
Carlton KS	1.3	17.7	0.15	6.0	0.02	—	—	23.7	0.17	12.8

<sup>a</sup>mg/cig; <sup>b</sup>mg/g of tobacco.

“tar” delivery by reducing the levels of the humectants added to the individual blends. A reduced humectant level in the blend would yield reduced levels of humectants in the TPM, thus reducing the FTC “tar” value. By utilization of eight cigarette design technologies categorized as significant (2), the FTC “tar” had been lowered from a sales weighted average of about 39 mg/cig in 1954 to about 14 mg/cig in the late 1970s.

In a 1979 study of the contribution of humectants to FTC “tar”, HEGE (15) determined the individual humectant deliveries in the MSS of a variety of commercial cigarette brands. He found that the “tars”, as determined by the FTC procedure, from Winston, Winston Lights, Camel Lights, Real, Salem, and Carlton consisted of 11–13% humectants, whereas the “tar” from Marlboro, Marlboro Lights, and Merit consisted of 21–26% humectants. For the Now, 17% of the “tar” consisted of humectants. At that time, Philip Morris cigarette products, in addition to glycerol and propylene glycol, contained triethylene glycol as a component of the humectant system. The data obtained by HEGE on humectants in various tobacco blends and their MSSs are summarized in Table 6. Later it will be seen that the humectants in MSS and CSC are effective biologically inactive diluents and reduce the biological activity of the condensate in mouse skin-painting studies and mutagenicity assays.

From a study to determine the effect of humectants added to tobacco on the FTC “tar” delivery, SWICEGOOD (96) reported in 1983 that the “tar” delivery of the 16-mg “tar” Camel Filter increased by 0.58 mg for each 1% increase in the amount of propylene glycol added to the cigarette tobacco. For this cigarette, 5.3% of propylene glycol and 7.7% of glycerol added to the tobacco transferred to the MSS. Glycerol, its level kept constant throughout the study of cigarettes with increased propylene glycol level, averaged 13.2% of the “tar” weight.

For Vantage and Winston Lights, the FTC “tar” contained an average of 1.4% and 1.0% propylene glycol, respectively, and 8.8% and 6.7% glycerol, respectively. The

transfer of glycerol from the tobacco to the MSS was 2.3% for Vantage and 1.8% for Winston Lights; corresponding data for propylene glycol were 4.5% and 4.0%, respectively (97).

More recent studies (98,99) with radiolabeled humectants (propylene glycol, glycerol) indicated that a portion of the humectants migrate to the filter tip and provide some removal by selective filtration of MSS components (100) in the same manner as do the plasticizers triacetin and Carbowax®. In contrast to other published reports, BEST *et al.* (102) and BEST (102) found no evidence for either the build-up of glycerol in the cigarette butt or elution of glycerol from the tobacco rod during smoking.

For many years, considerable thought had been given to the development of an accurate analytical method to determine the contribution of trace levels (a few µg/g of tobacco blend) of flavorants added to the cigarette tobacco to the levels of allegedly harmful components in tobacco smoke. Limitations of the analytical methodology precluded the design of an experiment whose results would be meaningful. It was recognized as recently as the late 1970s that even experiments with radiolabeled compounds had their limitations in the study of the pyrogenesis of MSS components (cf. SCHMELTZ *et al.*).

Although chemical data for the pyrogenesis of allegedly harmful smoke components from flavorants added to the blend at microgram levels are generally not available because of the above-mentioned limitations of analytical methodology, indirect confirmation of the effect of such additives on at least one MSS property is available; namely, the effect of addition of a total flavor formulation to the tobacco blend on the mutagenicity, as measured in the Ames *Salmonella typhimurium* test system, of the MSS particulate matter collected on a Cambridge filter pad.

Despite the use of humectants (glycerol, propylene glycol, etc.) as tobacco additives for many years, they attracted very little criticism. However, with escalation of the market in low- and ultralow-“tar” cigarettes, many blend components were examined for mutagenicity in the Ames test.

**Table 7. Experiment design: flavorants, casing materials, and humectants**

Cigarette <sup>a</sup> variation	Flavorant formulation level	Casing <sup>b</sup> and humectants <sup>c</sup> level
A	Usual level used on brand	Usual level used on brand
B	Ten times the usual level used on brand	0
C	0	Usual level used on brand
D	0	0

<sup>a</sup>Cigarette brands included Winston KS, Salem KS, Vantage KS, Camel Filter KS, and NOW KS manufactured in early 1977.

<sup>b</sup>Licorice, cocoa, and sugars.

<sup>c</sup>Glycerol and propylene glycol.

Among these were the humectants (glycerol, propylene glycol) actually used by RJRT as well as other humectants considered for use (triethylene glycol). Not only were the humectants examined “neat” as individual compounds but also they were examined as the formulations actually used or under consideration for use. In 1979, Bio-Research Laboratories, Inc. evaluated for RJRT the humectants glycerol, propylene glycol, and triethylene glycol and the humectant systems 2:1 glycerol:propylene glycol (the ratio of these humectants used in RJRT products) and 8:6:3 glycerol:propylene glycol:triethylene glycol (an humectant system occasionally considered for use in RJRT products) (104). The conclusions with respect to the compounds and systems tested were as follows:

Six samples . . . were submitted to Bio-Research Laboratories, Ltd. for assessment of mutagenicity in a plate incorporation assay system. At maximum tolerated doses, none of the compounds were mutagenic towards any of the five tester [*Salmonella*] strains <sup>a</sup>.

<sup>a</sup> The five *Salmonella typhimurium* tester strains were TA98, TA100, TA1535, TA1537, TA1538.

In this study and in other Ames bioassays conducted on samples submitted to Bio-Research by RJRT, positive controls such as *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (without liver homogenate) and 2-acetylaminofluorene (with liver homogenate) were used.

As an alternate to the arduous, expensive, and almost insurmountable task of studying individually the effect of the casing materials, humectants, and several hundreds of flavorful additives used in RJRT cigarette products, an experiment was devised that would show the effect on smoke condensate mutagenicity of the additives used in commercially available RJRT brands. These additive formulations were qualitatively and quantitatively unique for each commercial brand and the flavorant portion comprised as many as 70 different individual ingredients. The total weight of material in the flavor formulation added was of the order of 1.0 to 1.5 mg/g of tobacco blend. The design of the experiment involved the fabrication of four sets of cigarettes for each of five RJRT brands. Their levels of flavorants (“top dressing”), casing materials, and humectants were varied as shown in Table 7.

The MSS TPM from each of these four cigarette variations

**Table 8. Summary of mutagenicity data from various cigarette condensates (CSCs) (105)**

RJRT Brand	Strain	Mutagenicity in revertant/plate <sup>a</sup>			
		A <sup>b</sup>	B	C	D
Winston KS	TA1538	200 <sup>c</sup>	224	218	213
	TA98	215	250	245	249
Salem KS	TA1538	197	203	230	281
	TA98	254	232	256	310
Vantage KS	TA1538	171	195	175	204
	TA98	241	235	223	256
Camel Light KS	TA1538	199	169	145	176
	TA98	255	267	222	248
Now KS	TA1538	174	185	217	198
	TA98	241	227	296	268

<sup>a</sup>For MS CSC at 500 µg/plate with *Salmonella typhimurium* for cigarette variation.

<sup>b</sup>See Table 7 for description of cigarette variations.

<sup>c</sup>Each value is the average of 10 replicate determinations.

for five RJRT brands (Winston KS, Salem KS, Vantage KS, Camel Filter KS, and Now KS) was examined for specific mutagenicity in the Ames test (TA1538 and TA98 strains of *Salmonella typhimurium*) under a contract with Bio-Research Laboratories Ltd., Pointe Claire, PQ, Canada. From the results (Table 8), it was concluded (105):

Although the mutagenic activities appeared to be similar, there were statistically significant differences in mutagenic activities among the sample. It appeared that generally samples A were slightly less and samples D were slightly more mutagenic than the other samples.

Because the response of the *Salmonella typhimurium* was linear from 0 to 500 µg/plate of added WTPM, mutagenicity in revertant/plate was tabulated for the WTPM dose level of µg/plate. This permitted comparison (see Table 7) of the four cigarette variations for each *Salmonella typhimurium* strains and for each of the five commercial brands (105).

When Variations A and D are compared, exclusion of all additives (flavorants, casing materials/humectants) generally resulted in an increase in specific mutagenicity. Removal of the flavorants only (Variation C vs. A) produced no significant changes in the observed specific mutagenicity. Omission of the casing materials/humectants but augmenting the flavorants addition 10-fold (Variation B vs. A) generally resulted in specific mutagenicity increases.

As noted previously, inclusion of humectants (glycerol, propylene glycol, and/or triethylene glycol) in the tobacco blend results in transfer of substantial amounts of these additives from the tobacco rod to the smoke (both MSS and SSS).

Since these compounds are present in the WTPM (and FTC “tar”), it is not surprising that their removal from the additive system results in production of WTPM (and FTC “tar”) with increased specific mutagenicity in the Ames test. Both propylene glycol and glycerol – used in smoking products and demonstrated to be nonmutagenic either individually or combined (104) – act as diluents for the other WTPM components produced during the combustion process or transferred directly from the tobacco rod to the smoke during the smoking of the cigarettes.

Results from inhalation studies in rats exposed to propylene glycol (106) and other humectants (107) as aerosols indicated no significant adverse effect. LEE *et al.* (108) and DOOLITTLE and LEE (109) also reported that glycerol demonstrated no significant genotoxicity when examined in an *in vitro* test battery.

In a recent comparison of the MSSs from cigarettes containing glycerol and/or propylene glycol vs. the MSS from cigarettes containing no added glycerol or propylene glycol in a 13-week inhalation study in Fischer-344 rats, it was concluded that addition of the humectants being tested either singly or in combination had no meaningful effect on the site, extent, or frequency of respiratory tract changes associated with the smoke exposure in the test animals (110).

The casing materials and flavorants systems employed in the five RJRT brands do not appear, from these data, to impart increased mutagenicity to the MSS. In fact, their removal appears to increase slightly the observed mutagenicity of the WTPM. Presumably, the findings from this mutagenicity study indicate that the additives, including the flavorants formulations, do not contribute components to the smoke whose levels and/potency are such that they produce abnormal increases in the mutagenicity as measured in the Ames *Salmonella typhimurium* test system.

Although the results of a 1959 study indicated that the specific tumorigenicity of the CSC from cigarettes containing uncased tobacco was the same as that for the CSC from cased tobacco (111), no information was provided on the nature of the "casing materials" used. Since that time, little information has become available on the effect of increasing the humectant levels in the tobacco blend on the humectant content and/or the tumorigenicity of the MS CSC. It is known that the specific tumorigenicity (mouse skin-painting bioassay) and the B[a]P content of the CSC from a commercial cigarette gradually decreased between the mid-1950s and the early 1980s (112). Much of the decrease was attributed to the utilization of eight significant cigarette design technologies, but it is not known whether the humectant level in the FTC "tar" of the cigarette in question increased or decreased during the same period. If the humectant level did increase, the humectants may, as they did in the mutagenicity study, be acting as diluents for the tumorigenic components of the CSC.

#### 4 CONCLUSIONS

In the report by DOULL *et al.* (1) on the 599 ingredients that may be added to tobacco it was concluded:

Among those that pyrolyze, the pyrolysis products are not expected to depart significantly from those of additive-free tobacco.

It is important to recognize that the use of these ingredients has enabled manufacturers to develop cigarettes with lower "tar" and nicotine yields than would otherwise be available, and the primary issue in safety assessment is whether or not cigarettes are potentially hazardous as a result of the added ingredients. A careful analysis of the scientific data indicates that this is not the case . . .

It is concluded that the ingredients added to tobacco in the manufacture of cigarettes by the six major United States manufacturers are not hazardous under the conditions of use.

A detailed critique of the information available on the additives used primarily in casing materials formulations (sugars, cocoa, licorice) and as humectants (glycerol, propylene glycol, triethylene glycol) reinforces the conclusions presented by DOULL *et al.* These additives are the ones used at appreciable levels in the tobacco blend (0.5 to about 100 mg/g of tobacco).

Inclusion of modest levels of the casing materials (sugars, cocoa, licorice) and the humectants in the cigarette tobacco blend produces no serious variations in the chemical composition and/or the biological properties of the cigarette MSS that could be construed as potentially hazardous. In fact, data previously unpublished but currently available in various Federal and State repositories are presented to indicate that several of these additives may not only contribute to lower "tar" and nicotine deliveries but also may serve as diluents of suspect components of the "tar." The concern that glycyrrhizic acid, a pentacyclic component of licorice, may be a precursor of PAHs, particularly B[a]P, was shown to be unwarranted: Licorice generates much less B[a]P than does an equal weight of tobacco. The concern that added sugars may generate promoting phenols and tumorigens was offset by the finding that increasing the sugar level on tobacco reduces MSS mutagenicity. Because the humectants are nonmutagenic and represent a substantial portion of the FTC "tar", the mutagenicity of cigarette MSS WTPM in the Ames test is more or less inversely proportional to the humectants level on the tobacco and in its WTPM.

On the basis of this critique, it is concluded that the ingredients of the casing system (sugars, cocoa, licorice) and humectant system (glycerol, propylene glycol, triethylene glycol) added to tobacco during the manufacture of cigarettes are not hazardous under the conditions of use.

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## ERRATUM NOTICE

p. 284: left hand column, lines 12 and 13:  
For „The pipe and cigarette tobaccos yielded 27 and 10.5 mg of B[a]P” read “The pipe and cigarette tobaccos yielded 27 and 10.5 µg of B[a]P”