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Some Studies of the Effects of Additives on Cigarette Mainstream Smoke Properties. I. Flavorants*

by

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SUMMARY

Examination of extensive laboratory data collected during the past four decades, particularly considerable unpublished data generated between the mid-1950s and the late 1970s, indicates that none of the materials used as flavorants on smoking tobacco products, particularly cigarettes marketed by a US manufacturer, imparts any significant adverse chemical or biological properties to the mainstream smoke (MSS) from flavorant-treated tobacco, a conclusion reached by DOULL *et al.* (1) in their assessment of available information on nearly 600 ingredients variously used as cigarette tobacco additives in the US Tobacco Industry. In a more recent detailed assessment of the chemical and biological properties reported in the published literature for the MSS from cigarettes fabricated with tobacco with or without one or more additives, PASCHKE *et al.* (2) reached a similar conclusion; namely, that in general, no significant increase in the biological activity (carcinogenicity, mutagenicity, and cytotoxicity) of tobacco was reported from cigarettes containing added ingredients.

Many flavorful tobacco additives listed by DOULL *et al.* are structurally identical with or similar to highly polar, volatile components identified in the aqueous alcohol-soluble portion of cigarette MSS and tobacco. In the late 1950s, nearly two decades before the precise nature of the aqueous alcohol-soluble components of tobacco was defined, it was determined that their addition to cigarette tobacco produced no significant increase in the cigarette MSS of either the total polycyclic aromatic hydrocarbon (PAH) content or the benzo[*a*]pyrene (B[*a*]P) content, MSS components of considerable interest at that time. [Beitr. Tabakforsch. Int. 20 (2002) 83–103]

ZUSAMMENFASSUNG

Die Überprüfung umfangreicher Untersuchungsergebnisse der letzten vier Jahrzehnte, insbesondere eine beträchtliche Anzahl nicht publizierter Daten, die zwischen Mitte der fünfziger und den späten siebziger Jahren ermittelt wurden, weisen darauf hin, dass keine der Substanzen, die als Aromatisierungsmittel zu Tabakprodukten – insbesondere Cigaretten US-amerikanischer Hersteller – 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 Substanzen, die von der US-amerikanischen Tabakindustrie als Zusatzstoffe für Cigarettentabak benutzt wurden. In einer neueren detaillierten Übersicht der publizierten Literatur über die chemischen und biologischen Eigenschaften des HSRs von Cigaretten, die mit oder ohne Zusatz eines oder mehrerer Additive produziert wurden, kamen PASCHKE *et al.* zu einem ähnlichen Ergebnis: dass nämlich im allgemeinen bei Cigaretten mit Zusatzstoffen keine signifikante Erhöhung der biologischen Aktivität (Kanzerogenität, Mutagenität, Zytotoxizität) zu beobachten war.

Viele aromatisierende Tabakzusatzstoffe, die von DOULL *et al.* aufgeführt wurden, sind mit den hochpolaren, volatilen Inhaltsstoffen, die im wässrigen Alkohol-löslichen Anteil des HSRs von Cigaretten und im Tabak identifiziert wurden, strukturell identisch oder ähnlich. In den späten fünfziger Jahren, fast zwei Jahrzehnte bevor die genaue Natur der in wässrigem Alkohol-löslichen Inhaltsstoffe des Tabaks definiert wurde, fand man heraus, dass die Zugabe dieser Substanzen zum Cigarettentabak zu keiner signifi-

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kanten Erhöhung des Gesamtgehalts an polycyclischen aromatischen Kohlenwasserstoffen (PAHs) oder Benzo[*a*]-pyren (B[*a*]P) im HSR führte. Diesen Inhaltsstoffen des HSRs galt zu jenem Zeitpunkt besonderes Interesse. [Beitr. Tabakforsch. Int. 20 (2002) 83–103]

RESUME

L'examen des résultats obtenus au cours des quatre dernières décennies, et surtout les données considérables non-publiées du milieu des années 1950 jusqu'à la fin des années 1970, indiquent que les substances utilisées comme aromatisants des produits de tabacs – surtout des cigarettes produites aux Etats Unis – n'ont pas d'effets chimiques ou biologiques significativement défavorables sur les propriétés de la fumée du courant principal (CP) des tabacs auxquels ont été ajoutés ces aromatisants. C'est la conclusion à laquelle aboutissent DOULL *et al.* dans leur évaluation des informations disponibles sur presque 600 ingrédients utilisés comme additifs du tabac par l'industrie du tabac aux Etats Unis. Dans une évaluation détaillée plus récente de la littérature publiée sur les propriétés chimiques et biologiques du CP de cigarettes produites avec ou sans additifs, PASCHKE *et al.* aboutissent à une conclusion comparable: c'est à dire, qu'en général, on n'observe pas d'augmentation significative de l'activité biologique (carcinogénicité, mutagénité, cytotoxicité) des cigarettes contenant des additifs.

Beaucoup des aromatisants du tabac énumérés par DOULL *et al.* ont la même structure ou une structure similaire à celle des composants volatils fortement polaires, identifiés dans la partie soluble dans l'alcool aqueux du CP des cigarettes et du tabac. A la fin des années 1950, presque deux décennies avant la connaissance précise de la nature des composants solubles dans l'alcool aqueux, il a été déterminé que leur apport 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 suscitaient un intérêt considérable à cette époque. [Beitr. Tabakforsch. Int. 20 (2002) 83–103]

TOBACCO ADDITIVES

Having achieved greater "tar" reduction than the cigarette-smoking critics had originally proposed, for example see WYNDER (3), the Tobacco Industry unwittingly provided them with an alternate subject for criticism. The late 1970s, early 1980s heralded the advent of low-"tar" and ultralow-"tar" cigarettes and their acquisition of a significant share of the US cigarette market. Bases of the criticism were a) some commercial low-"tar" brands might have levels of additives much higher than the levels in previous high- and medium-"tar" cigarettes and b) the fates of many of the individual added components during the cigarette smoking process were unknown.

Such critical comments in the early 1980s about tobacco additives were not new. Earlier, concern was expressed about the pyrogenesis of PAHs from tobacco components (3) and their possible pyrogenesis from additives. In 1967, WYNDER and HOFFMANN wrote about additives (4):

> The importance of flavor-enhancing agents as contributors to the tumorigenicity in the experimental animals varies for different tobacco products. For cigarettes it may be a minor factor compared to the overwhelming effects of other constituents and variables. Nevertheless, one should emphasize that further studies on the toxicity of flavorants and their combustion products could provide a scientific basis for the selection of less harmful additives . . .
>
> 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.

However, the proponents of possible problems with tobacco additives became much more vocal about them when the nearly 70% reduction in sales-weighted MSS "tar" delivery between 1955 and 1985 not only answered the criticisms voiced in the late 1950s, early 1960s but met the goal set by others, i.e., the halving of "tar" delivery as a means to lower lung cancer incidence in cigarette smokers (3).

In 1980, LAVOIE *et al.* (5) wrote:

> The development of the low-tar, low-nicotine cigarette required cigarette fillers with a potential for smoke flavor contribution to make these cigarettes acceptable to the consumer. Such products can be realized either by selecting tobaccos rich in flavor or by addition of tobacco extracts or certain plant extracts, addition of synthetic flavor compounds, or a combination of several of these factors . . .
>
> New cigarettes should be assayed for toxicity and tumorigenicity, so that the reduction of toxic and tumorigenic effects in the smoke of low-tar, low-nicotine cigarettes is not offset by the introduction of unknown factors.

Despite their criticism of the possible increased use of flavorants in the filler of low-"tar", low-nicotine cigarettes, a key part of this discussion is the authors' admission that prior to 1980, the US cigarette manufacturers had, in their opinion, apparently achieved a "reduction of toxic and tumorigenic effects in the smoke of low-"tar", low-nicotine cigarettes".

In the 1979 Surgeon General's report (6) the following was written:

> the trend toward low-tar, low-nicotine cigarettes and toward a reduction of undesirable volatile smoke compounds has brought about major changes in the smoke flavor of cigarettes. The use of rolled stems and reconstituted tobacco sheet admixed with leaf lamina and the use of effective filter tips are major factors inducing changes in smoke flavor. All of these developments have led to increased use of flavor additives, especially for low-tar, low-nicotine cigarettes. In fact, these new cigarettes require flavor corrections by additives in order to be acceptable to the consumer. Tobacco extracts as well as nontobacco flavors, such as licorice,

> cocoa, fruit, spices, and floral compositions, are used . . . At present, the selection of tobacco flavor additives from the GRAS (Generally Regarded As Safe) List or from natural extracts and the screening of their smoke decomposition products for toxicity or other biological activity are not required by law and are done voluntarily by manufacturers.

Temperatures to which flavorants added to tobacco are exposed and the duration of the exposure during the smoking process range from 500 to 700 ˚C and the few seconds of the puff duration, respectively. Many of the flavor additives listed by the Surgeon General are used in cooking and/or baking where the exposure temperatures are lower than in the smoked cigarette but the exposure time to the elevated temperature is much longer. This raises the question: Will more toxic compounds be formed from a given flavorant during foodstuff preparation or during cigarette smoking? When questioned about the need to determine the generation of toxic substances from a GRAS list additive used in cooking and/or baking, the Food and Drug Administration (FDA) stated such studies were not required of the foodstuff manufacturer nor could they be done by the FDA since it had neither the staff, facilities, nor funds to undertake such studies.

It was noted in the Surgeon General's 1981 report (7):

> Humectants and flavoring agents have long been used as additives in cigarette manufacture . . . In recent years, cigarette manufacturers' advertisements have focused on the flavor of new lower "tar" and nicotine cigarettes, enhanced presumably by the addition of tobacco constituents or by the addition of new flavoring materials, such as natural and synthetic chemicals. The identities and amounts of the additives actually used in the manufacture of U.S. cigarettes are not known. Systematic information has not been published or made available on the influence of these additives on the composition or biological activity of cigarette smoke.

Essentially the same sentiments were noted in 1982 (8):

> The development of the low-tar cigarette required enrichment of smoke flavors in order to make the product acceptable to the consumer. The flavor is enhanced by addition of undescribed materials that may include concentrates of flavor precursors obtained from tobacco, licorice, extracts from other plants, or semisynthetic or fully synthetic flavor components. Because these additives have not been identified, no judgment can be made as to whether they result in new compounds or higher concentrations of hazardous components in the smoke. The practice of flavor enrichment requires detailed toxicological studies that are not available at present for scientific evaluation of their impact [LaVoie *et al*. (5); United States Public Health Service (6)].

In reports issued in 1994 by DOULL *et al*. (1) and in 2000 by PASCHKE *et al*. (2) on detailed analysis of literature reports on the effects of ingredients added to cigarette tobacco on the chemical and biological properties of its MSS, both groups essentially reached the same conclusion: Both groups concluded that the added ingredients under the conditions of use contributed no adverse chemical or biological properties to the MSS.

In their 1997 review of the changed cigarette, HOFFMANN *et al*. (9) did not discuss low-"tar" cigarettes or the presumed use of additional flavoring materials, identity unknown. In a second 1997 article on changes in cigarette design implemented between 1950 and 1995, HOFFMANN and HOFFMANN (10) discussed casing materials and flavor additives. They discussed the casing additives sugars and humectants (glycerol, propylene glycol, diethylene glycol) but failed to mention that some cigarette manufacturers do not use diethylene glycol. With regard to humectants, their transfer to cigarette MSS and their significant contribution to the Federal Trade Commission (FTC) "tar" value were ignored. On flavor additives, they wrote:

> In April 1994, the major U.S. cigarette companies released a list of 599 additives used at that time for the manufacture of cigarettes [Doull *et al*. (1)]. However, in the past, additional reactive flavor additives have been used (such as angelica lactone and linalool oxide; Leffingwell (11)). An exception is menthol, which amounts to less than 2.5 mg in U.S. mentholated cigarettes [Perfetti & Gordin (12)]. Menthol is not carcinogenic in rodents [National Cancer Institute (13)], nor does this readily volatilized compound give rise to measurable amounts of carcinogenic hydrocarbons during smoking of cigarettes [Jenkins *et al*. (14)]. Yet it is possible that the spraying of tobacco with menthol affects the burning characteristics of a cigarette and thus changes the concentration of toxic and/or tumorigenic agents in the smoke.

The HOFFMANNS obviously ignored what WYNDER and HOFFMANN (15) wrote about the findings of BOCK *et al*. (16) on the specific tumorigenicity of the MSS from menthol cigarettes:

> The results of Bock *et al*. [16] suggest no difference in tumorigenic activity of heptane-soluble "tar" from a mentholated cigarette compared with a plain cigarette when tested on a gram-to-gram basis.

Materials added to the tobacco blend during its preparation for inclusion in the final cigarette are generally classified as flavorants, casing materials, and humectants.

- *Flavorants:* Flavorants added to cigarette tobacco blends include a) menthol which may be used at a level as high as 0.8% (8 mg/g) of the final tobacco blend weight and b) a variety of materials, possibly numbering as many as 100, the total weight of which does not exceed 0.2% (2 mg/g) of the tobacco blend weight. 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 by most consumers.
- *Casing materials* (17): These include sugars, licorice, and cocoa which have been used for many years in the cigarette tobacco blend, the so-called American tobacco blend, whose first prototype was a blend of flue-cured, burley, and Oriental tobaccos in the Camel 70-mm cigarette introduced by R. J. Reynolds Tobacco (RJRT) Company in 1913.
- *Humectants*: Humectants traditionally used in cigarette manufacture are glycerol and propylene glycol. Triethylene glycol is also used as an humectant by some cigarette manufacturers.

The major topic in this report is the flavorful materials added to the tobacco filler and their effect on MSS properties. In a companion report (18), the effects of casing materials (sugars, cocoa, licorice) and humectants (glycerol, propylene glycol, etc.) on MSS properties are discussed.

TOBACCO ADDITIVES: FLAVORANTS

More than 1100 materials have been proposed (19) in the scientific literature or in US patents for use as tobacco additives to impart consumer-acceptable taste and/or aroma characteristics and other properties to the product and/or its smoke. Most of these proposed materials are highly flavorful. However, their listing does not imply that all are used in cigarette manufacture. 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 (17). Many "top-dressing" components are structurally identical with or similar to identified tobacco components. With no evidence to the contrary, it is assumed that such an individual added flavorant 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 flavorant formulation, usually unique for each brand, may comprise as many as 100 flavorful materials (plus menthol in the case of a mentholated cigarette) and is added a) to improve the aroma and taste of the cigarette MSS and the aroma of its SSS and b) to provide a pleasant "pack aroma", particularly when the cigarette pack is first opened by the consumer.

Despite the number of components in the "top dressing", their total weight seldom exceeds 2 mg/cig. That is, a component in the "top dressing" comprising 100 components is initially present in the blend at a level, on average, of 20 μg/cig (17). Many of these flavorants are relatively volatile, usually of low or moderate molecular weight and, of course, are highly flavorful. Because of their volatility, the levels of many of these flavorants on the tobacco filler gradually decrease after the cigarette pack is opened.

Menthol is usually added at a level much higher than that of the flavorants that constitute the "top dressing". Its level may be as high as 0.8% of the tobacco filler weight, i.e., 800 mg of mentholated cigarette tobacco filler might include 6.4 mg of menthol. In the sealed cigarette pack, menthol rapidly equilibrates between the filler and the filter tip (20). The equilibrium is dependent on the nature and level not only of the plasticizer (triacetin, Carbowax®, etc.) but also of other filter-tip additives (charcoal, treated charcoal) used in the filter tip (21).

The study of tobacco additives and their contribution to smoke composition and properties provides an excellent example of the significance of analytical methodology on our ability to generate meaningful data on the relationships between tobacco components, added components, and smoke components. The contribution of individual flavorants to MSS and SSS properties is much more difficult to study than is the contribution of casing materials and humectants because the latter are usually added at a much higher level than the individual flavorants (excluding menthol).

As reported previously, considerable capability was available to determine the contribution of various additives to the PAH content of cigarette MSS (22). To determine the fate during the smoking process of 10 or 20 μg of a particular compound when either the compound itself or its reaction products may be distributed among the particulate and vapor phases of the MSS and SSS is a tremendous analytical challenge.

Because of this analytical problem with additives to the cigarette filler, many investigators utilized pyrolysis of individual tobacco components or additives in an attempt to define the spectrum of products and their influence on tobacco smoke composition and properties.

In both instances, a given compound may undergo a variety of reactions: In the pyrolysis case, fragments produced from the compound during pyrolysis only have the opportunity to react with the unchanged compound itself or with each other. In the smoked cigarette case, the added compound itself or fragments produced from it during the smoking process have the opportunity not only to react with intact volatilized tobacco components (over 3600 of which have been identified [23]) but also to react with the reaction fragments produced from them.

If it is assumed that a given compound, Compound *X*, during pyrolysis is not only transferred in part to the pyrolysate but also yields three pyrosynthetic fragments (*A*, *B*, and *C*), then these four entities (*X*, *A*, *B*, and *C*) may transfer to the pyrolysate intact or interact in a variety of ways to form a mixture of pyrolysate components:

$$X \longrightarrow X + A + B + C \xrightarrow[\text{plus transfer of intact } X]{\text{entities interact with one another}} \text{pyrolysate}$$

If it is assumed not only that the same type of reaction occurs in a cigarette during the smoking process in the case of Compound *X*, either added to or inherent in the tobacco blend, but also that similar reactions occur with the other tobacco components (X_1, X_2, X_3, …, X_n) the situation described in the following equations could exist, where *n* could be as high as or higher than 3600, the approximate number of identified tobacco components:

$$\begin{aligned} X &\longrightarrow X + A + B + C \\ X_1 &\longrightarrow X_1 + A_1 + B_1 + C_1 \\ X_2 &\longrightarrow X_2 + A_2 + B_2 + C_3 \\ X_3 &\longrightarrow X_3 + A_3 + B_3 + C_3 \\ \cdots &\quad \cdots \\ X_n &\longrightarrow X_n + A_n + B_n + C_n \end{aligned} \xrightarrow[\text{plus transfer of intact } X, X_1, X_2, X_3, \ldots, X_n]{\text{entities interact with one another}} \text{cigarette smoke}$$

In this case, Compound *X* and its fragments *A*, *B*, and *C* have the opportunity to react with each other as well as to interact with $X_1, X_2, X_3, \ldots, X_n$; $A_1, A_2, A_3, \ldots, A_n$; $B_1, B_2, B_3, \ldots, B_n$; and $C_1, C_2, C_3, \ldots, C_n$. In both the pyrolysis case and the

Table 1. Comparison of polycyclic aromatic hydrocarbon fraction levels, phenol yields, and carboxylic acid yields in 700 ˚C pyrolysates from tobacco, petroleum ether extractables (PEE), and the tobacco residue (RES) after extraction

Pyrolysate components	Amount from Tobacco (µg/1000 mg)	PEE (µg/80 mg)	RES (µg/920 mg)	Total PEE + RES (µg/1000 mg)	% Difference (total vs. tobacco)
Polycyclic aromatic hydrocarbon group					
Naphthalene	3200	3900	1300	5200	63
Fluorene	1100	1100	860	1960	78
Phenanthrene	1600	1500	780	2280	43
Pyrene	630	820	350	1170	86
Chrysene	180	260	80	340	89
Benzopyrene	190	140	50	190	0
Totals	**6900**	**7720**	**3420**	**11,140**	**61**
Phenol group					
Phenol	3610	50	2620	2670	−26
o-Cresol	750	50	630	680	−20
m-/*p*-Cresol	1620	50	1180	1230	−24
Ethyl-/dimethylphenols	910	130	700	830	−9
1-Naphthol	160	30	110	140	−13
2-Naphthol	140	20	110	130	−7
Totals	**7190**	**330**	**5350**	**5680**	**−21**
Acids					
Volatile acids (formic through heptanoic)	330	140	230	370	12
Nonvolatile acids (undecanoic through tetratriacontanoic)	12	11	4	15	25
Totals	**342**	**151**	**234**	**385**	**13**

cigarette smoke formation case, the number of fragments may, of course, be many more than the three designated as *A*, *B*, and *C*.

Obviously, pyrolysis of an individual compound (Compound *X*) at a specific temperature and during the smoking process in a machine-smoked cigarette whose blend contains Compound *X*, either added or inherent, are entirely different situations and will yield qualitative and quantitative differences between the compositions of the pyrolysates and the cigarette smoke. Qualitatively there may be some similarities in the two compositions. Quantitatively, the probability of any similarity is extremely low. It should be noted that during the pyrolysis of Compound *X*, a specific temperature such as 700 ˚C or 600 ˚C is maintained. During the smoking process occurring in the Compound *X*-containing cigarette, Compound *X* and its pyrogenetically generated fragments are exposed to a range of temperatures varying from nearly 1000 ˚C at the fire cone to 50 to 60 ˚C near the butt. In addition, BRITT *et al.* (24) noted that the residence time during most pyrolysis studies of tobacco components was much longer than that encountered by the tobacco components during the smoking process.

The dilution effect described above has been demonstrated to be real, not however in a smoke formation study with a cigarette but in a pyrolysis study. In 1978, SEVERSON *et al.* (25) reported the results of a study of the pyrolysis of tobacco, petroleum ether extractables (PEE: 8%) of the tobacco, and the tobacco residue (RES: 92%) after the extraction. Each of these three materials was pyrolyzed at 700 ˚C in an N_2 atmosphere, conditions which SEVERSON *et al.* claimed would yield tobacco pyrolysates whose PAH profiles could be correlated with cigarette smoke condensate (CSC) PAH profiles.

In Table 1, adapted from (25), 1000 mg is used to approximate the weight of filler in some cigarettes. While no attempt was made to explain the divergent phenol and acid data, SEVERSON *et al.* explained their PAH findings as follows:

> In agreement with previous work [26], the PE extract produced the most PAH. The 8 g of extract (8%) yielded 2 to 3 times as much PAH as the 92 g (92%) of the [residue]. The sum of the weights of the PAH produced separately by the PE extract and the [extracted tobacco residue] was considerably higher than the amount produced by the whole tobacco. This is readily explained by the mechanisms proposed by Badger *et al.* [27] and Jones *et al.* [28] . . . When tobacco is pyrolyzed, the PAH-producing intermediates, resulting from the decomposition of PE solubles, are diluted with other non-PAH-producing products so that the probability of a PAH-forming bimolecular collision decreases. As a result, the amount of PAH produced during tobacco pyrolysis decreases.

Over the years, considerable thought was given by RJRT personnel to a method to determine the contribution to cigarette MSS properties of trace levels (from less than a few µg/g to about 4 mg/g of cigarette filler) of flavorful components added to tobacco. The high level refers to menthol.

Table 2. Modification of smoke composition by removal of tobacco components by solvent extraction or by addition of tobacco components

Year	Investigator(s)	Effect on		Comments
		Level of PAHs	Biological activity[a]	
1942	ROFFO (32)	decreased	decreased	"Tars" were not generated by a smoking process but were generated unrealistically by "destructive distillation" of control and solvent-extracted tobacco.
1955–1958	ASHBURN (36); RODGMAN (35); RODGMAN and COOK (37)	decreased	—	Solvent extraction of all tobacco types and blends *decreased* the levels of PAHs in MS CSC. Extraction removed wax-like materials such as the saturated aliphatic hydrocarbons, phytosterols, solanesol, duvane derivatives (74).
				Addition of the wax portion from the extract to the extracted tobacco *increased* the PAH level in MS CSC to that found in the control tobacco MS CSC.
				Addition of the aqueous ethanol-soluble portion of the extract to the extracted tobacco produced *no change* in the PAH levels of the MS CSC vs. those found in the extracted tobacco MS CSC.
				Addition of both the waxes and the aqueous ethanol-soluble portion from the extract to the extracted tobacco *increased* the PAH level in MS CSC to a level slightly greater than that of the control tobacco MS CSC.
				Results indicated polar components (highly soluble in aqueous ethanol) of tobacco are *not* major precursors of MSS PAHs.
				Addition of saturated aliphatic hydrocarbons or phytosterols or solanesol *increased* PAH levels in the treated tobacco MS CSC over that from the control tobacco MS CSC. Changes in levels of individual PAHs were *not* uniform. This result indicated B[*a*]P is not really a valid "marker" or "indicator" for tumorigenic tetracylic and higher PAHs.
				Organic solvent extraction of tobacco not only *decreased* the levels of PAHs in cigarette MSS PAHs but also *increased* the MSS levels of phenols (phenol, *o*-cresol, *m*-cresol, *p*-cresol), aldehydes, and ketones.
1956	CAMPBELL and LINDSEY (75)	decreased	—	Changes in levels of different PAHs were not uniform and did not parallel the change in level of B[*a*]P.
1956	WYNDER (76)	—	decreased	Preliminary announcement of effect of extraction on MSS chemistry and biological properties.
1957–1958	RAYBURN and WARTMAN; RAYBURN *et al.* (77)	no change	—	Tobacco saturated aliphatic hydrocarbons or an individual ^{14}C-labeled hydrocarbon added to tobacco prior to smoking did not produce a significant increase in levels of PAHs or B[*a*]P in cigarette MS CSC.
1957	WRIGHT (78)	—	decreased	Wright considered the phytosterols and terpenoids to be the major precursors in tobacco of PAHs in MSS.
1958	WYNDER *et al*. (79)	decreased	decreased	Authors demonstrated that pyrolysis of tobacco phytosterols yielded higher PAH levels than tobacco saturated hydrocarbons pyrolyzed under the same conditions (cf. [80]).
1959	WYNDER and HOFFMANN (81)	decreased	decreased	Decrease in % tumor-bearing animals skin-painted with MS CSC from organic solvent-extracted tobacco was less than % decrease in levels of PAHs (including B[*a*]P) in the cigarette MS CSC.
1960	WYNDER and HOFFMANN (82)	decreased	decreased	Authors ruled out solvent extraction of tobacco as realistic means to reduce PAHs in cigarette MS CSC and its specific tumorigenicity because the process was "impractical both technically and economically".
1960	HAEFELE and GILES (83)	—	—	Pyrolysis products from radiolabeled *n*-hentriacontane included PAHs.
1961	RODGMAN and COOK (84)	decreased	—	Levels of the simple phenols (phenol, *o*-cresol, *m*-cresol, *p*-cresol) in MSS from cigarettes made with organic solvent-extracted tobaccos were *increased* over levels in control tobacco MSS.
1962	NEUKOMM and BONNET[b] (85)	decreased	—	Organic solvent-extraction of tobacco resulted in *decrease* of PAHs in cigarette MS CSC. US patent issued to Bonnet and Neukomm on solvent extraction process.

Table 2 (contd.)

Year	Investigator(s)	Effect on		Comments
		Level of PAHs	Biological activity[a]	
1963	CUZIN *et al*. (86)	no change	no change	An unsuccessful attempt to duplicate the MSS PAH findings with tobaccos solvent-extracted by the Neukomm-Bonnet process.
1963	UHLMANN (87); NICOD (88)	no change	no change	Uhlmann examined MS CSC from tobaccos extracted by the Nicod solvent-extraction process
1964–1968	WYNDER and HOFFMANN (89); HOFFMANN and WYNDER (90)	decreased/ no change	decreased/ no change	Review of published organic-solvent extraction studies and discussion of PAH levels per cigarette, per gram of MS CSC, etc. and effect of CSC painting on % tumor-bearing animals in mouse skin-painting experiments.
1976	WYNDER and HECHT (91)	decreased	decreased	The authors described the organic-solvent extraction of tobacco as a means to remove PAH precursors from tobacco and reduce levels of PAHs in MS CSC as only being "*of academic interest*".
1979	US Surgeon General (6)	decreased	decreased	The Wynder-Hecht table listing successful means to reduce MSS "tar", B[*a*]P, and CO monoxide and specific tumorigenicity (mouse-skin painting) was reproduced in this Surgeon General's report.

[a]Sebaceous gland suppression test or mouse skin-painting study.
[b]This was a patent similar to that applied for by RODGMAN and ASHBURN on behalf of R. J. Reynolds Tobacco Company RJRT patent application was approved but never issued. However, similar patents were issued to RJRT in Italy and France in 1959 and 1960, respectively (74).

Government agencies such as FDA are mainly concerned with the toxicity of compounds added to consumer goods but have neither the funds nor personnel to assess the fate of most additives when heated. Flavorants and other additives in a cake mix are approved for use on the basis of satisfactory toxicity data derived from tests on the additives "as is". What happens to them during the lengthy baking period at elevated temperatures is generally not considered by government agencies. Much information is available on the generation of tumorigenic PAHs, *N*-nitrosamines, and *N*-heterocyclic amines by exposure of foodstuffs to the temperatures and times usually used in manufacture, cooking, and/or baking (see MAGA, 29).

Flavorants, casing materials, and humectants added to cigarette tobacco are subjected to temperatures ranging from ambient to high (>700 °C) for a brief time period (30). Most flavorants are relatively volatile, from low to moderate molecular weight, and are added to the tobacco blend at microgram (ppm) levels. Radiolabeled flavorants could be used, but many would require special synthesis to insert one or more radiolabeled centers. Most compounds used as flavorants in tobacco smoking products are not available commercially as radiolabeled compounds, a situation similar to that noted by SCHMELTZ *et al.* (31):

> However, studies such as these have limitations because many tobacco constituents (e.g., terpenoids) containing label are hardly accessible. Moreover, those that are available are usually not of sufficient activity for tracer studies or are labeled in only one position.

If the radiolabeled flavorants were available or readily synthesized, the magnitude of the effort required to conduct fate studies on over 450 individual compounds (1) and to determine their contribution to the nature and levels of smoke components (phenols, aldehydes, ketones, *N*-nitrosamines, PAHs, *N*-heterocyclic amines, etc.) or to the levels of allegedly harmful members within these classes would be astronomical.

Table 2 summarizes representative publications issued between the mid-1950s and late 1970s plus the 1942 report by ROFFO (32) on studies conducted in attempts to define the precursors in tobacco of specific allegedly harmful components in cigarette MSS.

From the mid-1950s to the early 1980s, the majority (about 95%) of the investigations of the effect of either removal or addition of tobacco components on cigarette MSS composition dealt with those components whose properties overall are significantly different from those of compounds and materials usually classified as "flavorants" or "top dressing" components (17).

The major chemical and physical differences between these "flavorants" and the precursors studied over the past three decades are summarized in Table 3. Molecular weight and volatility are the most significant properties in the behavior differences between "flavorants" and precursors. Obviously, in a complex system such as tobacco – comprising at least 3600 identified components (23) – a few exceptions to the rules may be cited. In general, these properties – molecular weight and volatility – are closely related: The higher the molecular weight, the lower the volatility, hence the greater the opportunity for reaction within the tobacco rod of the burning cigarette.

These differences permit a logical explanation why "flavorants" have a low probability of involvement in the pyrogenesis of allegedly harmful smoke components such as the PAHs. The volatilities of the "flavorants" enable a large proportion of them to escape rapidly from the higher

Table 3. Major differences between "flavorants" and precursors in tobacco of allegedly harmful smoke components

Flavorants	Precursors
Molecular weight is relatively low; i.e. generally less than 200.	Molecular weight is generally relatively high, i.e., generally greater than 200. In fact, major precursors of some classes of MSS and SSS components are polymeric. For example, lignin, cellulose, pectins, starch, and proteins are natural biopolymers with extremely high molecular weights which preclude their volatilization during the smoking process. The major precursors of the PAHs include solanesol (mol. wt. > 600) and its esters, the phytosterols (mol. wt. > 400) and their esters, and the saturated aliphatic hydrocarbons (mol. wt. ranging from 282 for $C_{20}H_{42}$ to 562 for $C_{40}H_{82}$, etc.)
Moderate to high volatility under temperature conditions existing in the tobacco rod during the smoking of the cigarette.	Relatively low volatility under conditions existing in a burning cigarette. Some smoke component precursors decompose to simpler and lower molecular weight entities during the smoking process. These, in turn, may undergo additional reactions. For example, tobacco proteins decompose to amino acids which subsequently could yield *N*-heterocyclic compounds; celluloses, pectins, and starch decompose to simple sugars which, in turn, could yield aldehydes, ketones, and acids.

temperature zones in the tobacco rod of the smoking cigarette and to transfer intact to MSS during the puff and to SSS between puffs. Evidence for this was provided by the elegant pyrolysis studies with several specific flavorants by STOTESBURY *et al*. (33,34). In contrast, tobacco components with low volatilities reside for relatively prolonged periods of time in the higher temperature zones of the rod, conditions amenable to numerous reactions (dehydration, oxidation, reduction, decomposition and recombination of the fragments, decarboxylation, aromatization, cyclization, etc.).

On the basis of results from the following studies, the nature of the major precursors in tobacco of allegedly harmful smoke components was defined:

- Studies on the effect of non-polar organic solvent extraction of tobacco on cigarette MSS composition, with particular emphasis on PAH levels: Reduction of per cigarette deliveries of FTC "tar" and PAHs, but usually not to the same extent. Partition of the extract between non-polar (pentane, hexane) and polar (aqueous alcohol) solvent systems indicated that the PAH precursors resided primarily in the pentane or hexane fraction.
- Studies to define the composition of the material extracted by the non-polar solvent: The extracted material comprised saturated and unsaturated aliphatic hydrocarbons, phytosterols and their esters with long-chained fatty acids, long-chained aliphatic alcohols and terpenoid alcohols, e.g., solanesol, and their esters with long-chained fatty acids. Subsequently, duvanediols with 14-carbon rings (the number of carbons in anthracene and phenanthrene) were identified in the extract.
- Pyrolysis studies with the total extracted material and its individual components of classes of components: Pyrolysis of the materials (usually less than 10% of the tobacco weight) extracted by the non-polar solvent yielded about twice the weight of PAHs as did similar pyrolysis of the extracted tobacco (representing about 90% of the weight of the original tobacco) (25). All of the non-polar solvent-extractable tobacco components (saturated and unsaturated aliphatic hydrocarbons, solanesol, phytosterols, long-chained esters, etc.) on pyrolysis yielded relatively high levels of PAHs and are considered to be the prime precursors in tobacco of the PAHs in cigarette smoke.

In RJRT studies (cf. RODGMAN [35] and ASHBURN [36]), it was demonstrated that organic solvent extraction of individual tobacco types and various tobacco blends resulted in an extracted tobacco residue which, on smoking in cigarette form, yielded lower levels of PAHs in its MSS than did the original tobacco. In agreement with the findings of others, major precursors were shown to include the phytosterols, the terpenoid alcohol solanesol, and the saturated aliphatic hydrocarbons. Phytosterols and long-chained alcohols esterified with long-chained fatty acids were also shown to be precursors in tobacco of PAHs in smoke (cf. 25). These components were removed from tobacco to some degree by the various solvents (pentane, hexane, diethyl ether, etc.) used in the RJRT extraction studies.

An additional study (37) was conducted wherein tobacco-derived saturated aliphatic hydrocarbons, solanesol, and β-sitosterol were added individually at two levels to a commercial tobacco blend. Several other flavorful compounds (sclareolide, disodium salts of two substituted malonic acids) were also studied. The effect of the added compounds on the levels of total PAHs and several individual PAHs in MSS was determined. Because much of the early cigarette MSS research at RJRT was conducted in parallel with research at other laboratories, a smoking regimen similar to that described by WYNDER *et al*. (38) was used at RJRT in the 1950s to generate MSS data (39). The reason for the matching of the smoking regimen (smoking machine, collection system, smoking parameters, etc.) was the possibility of correlating the chemical composition data at RJRT with the biological data reported by WYNDER *et al*. The WYNDER *et al*. and the early RJRT smoking regimen involved puffing parameters of a 35-mL puff volume, 2-sec puff duration, 1 puff/20 sec vs. the smoking procedure proposed by BRADFORD *et al*. (40), a 35-mL puff volume, 2-sec puff duration, 1 puff/min. Based on the 1964 report by OGG (41), the BRADFORD *et al*. parameters were subsequently dictated as standard by the FTC in the United States (42). The assumption that the % increase in B[*a*]P for a given cigarette would be the same for both smoking regimens is not an unreasonable one. Results are summarized in Table 4, modified from RODGMAN and COOK (37). Inserted into Table 4 is a column

Table 4. Effect of added tobacco components and other organic compounds on levels of total polycyclic aromatic hydrocarbons and benzo[*a*]pyrene in cigarette mainstream smoke condensate

Additive	mg/g Tobacco	Increase of total PAHs		Increase of benzo[*a*]pyrene		
		%	%/mg	%	%/mg	%/10 µg
		To Winston Blend tobacco				
Solanesol[a]	3.2	15	4.7	13	4.1	0.041
Solanesol[a]	6.4	24	3.7	13	2.0	0.020
Saturated hydrocarbons[b]	2.0	24	12.0	13	6.5	0.065
Saturated hydrocarbons[b]	4.0	44	11.0	20	5.0	0.050
β-Sitosterol[c]	1.9	26	13.7	16	8.4	0.084
β-Sitosterol[c]	3.8	41	10.8	28	7.4	0.074
Mineral oil	4.0	13	3.2	5	1.2	0.012
Trimyristin[d]	4.0	6	1.5	6	1.5	0.015
Cystine	1.00	6	8.0	ND[e]	—	—
Methionine	1.25	6	4.8	ND[e]	—	—
		To RTS[f]				
Sclareolide[g]	4.5	7	1.6	6	1.3	0.013
Saturated hydrocarbons[b]	4.0	48	12.0	27	6.7	0.067
Disodium isopropylmalonate[h]	3.0	(1)	(0.1)	3	0.4	0.004
Disodium *sec*-butylmalonate[h]	4.5					

[a]Isolated from tobacco; mol. wt. = 630.
[b]Isolated from tobacco; average mol. wt. = 400.
[c]Identical with β-sitosterol isolated from tobacco (and smoke); mol. wt. = 414.
[d]Synthetic glyceride; mol. wt. = 722.
[e]The RTS was G7X, an RJRT proprietary reconstituted tobacco sheet.
[f]Isolated from Oriental tobacco; mol. wt. = 250.
[g]Added as a mixture of the two disodium salts of the alkylmalonic acids; total amount added = 7.5 mg/g (3.0 + 4.5 mg/g) of G7X.
[h]ND = not determined.

of data, calculated from the original data, which show the % increase in B[*a*]P per milligram of precursor added to the tobacco. This "spiking" study, particularly the β-sitosterol experiment, provided data on the chrysene:B[*a*]P ratio in the cigarette MSS which demonstrated that B[*a*]P was not an "indicator" of other tumorigenic tetracyclic PAHs in MS CSC, a finding later confirmed in the National Cancer Institute (NCI) "less hazardous" cigarette study where the MSS B[*a*]P level was found not to be an "indicator" of the MSS B[*a*]A level (43). Chrysene is a well-known thermal degradation product of sterols, including the phytosterols (Figure 1).

Examination of the structure of the tricyclic sclareolide, one of two major contributors to the unique flavor and aroma of Oriental tobacco smoke, reveals that, theoretically, two molecules could combine, lose carbon dioxide and methyl groups, and undergo aromatization to yield dibenz[*a*,*h*]anthracene (DBA) (Figure 2). To determine whether this occurred, a "spiking" experiment was conducted with sclareolide to assess its contribution to the DBA level in cigarette MSS (37). While increasing the sclareolide level to many times that normally present in Oriental tobacco or in a typical commercial blend containing 15% or so of Oriental tobacco did produce a slight increase in total PAHs, including B[*a*]P, in the MSS (Table 4), no increase in MSS DBA was observed.

Most tobacco additives used at RJRT were GRAS (Generally Regarded as Safe) and/or FEMA (Flavor and Extract Manufacturers Association) approved materials. However, concern over the safety of additives used on RJRT smoking products occasionally prompted studies to resolve unsubstantiated literature claims or rumors in the press. For example, in the 1950s and early 1960s, the flavor and aroma of cigarette MSS were enhanced by addition to tobacco of coumarin (2*H*-1-benzopyran-2-one), an extremely low-level tobacco component. The question was raised: Did coumarin, during the smoking process, yield dicumarol (Figure 3), a potent anti-coagulant[1]? In 1963, NEWELL (44) demonstrated with ^{14}C-labeled coumarin that cigarettes fabricated from tobacco containing ^{14}C-radiolabeled coumarin yielded no ^{14}C-radiolabeled dicumarol in the MSS.

A flavorant not on the GRAS or FEMA list and not identified in tobacco or tobacco smoke is the ester tetraisovalerylglucose, an homolog of acetyl-*tris*-(3-methylvaleryl)glucose, an Oriental tobacco isolate identified by SCHUMACHER (45) and subsequently identified in Oriental tobacco smoke (46). During smoking, acetyl-*tris*-(3-methylvaleryl)glucose yields 3-methylvaleric acid at high levels, a characteristic of Oriental tobacco smoke which differentiates it from burley, Maryland, or flue-cured tobacco smokes. The levels of 3-methylvaleric acid in the tobaccos themselves also parallel the smoke findings (47). The level of this acid in various Oriental tobaccos is as much as 70 to 100 times that in flue-cured, burley, or Maryland tobaccos. Attempts to identify the readily synthesized tetraisovaleryl homolog in Oriental tobacco were unsuccessful even though other homologs

[1]The source of concern about the coumarin-to-dicumarol conversion was initiated by reports that cattle consuming moldy plant material, particularly sweet clover hay, in which coumarin had been converted to dicumarol were subjected to uncontrollable hemorrhaging if their feed contained material (thistles, etc.) that punctured their digestive tract.

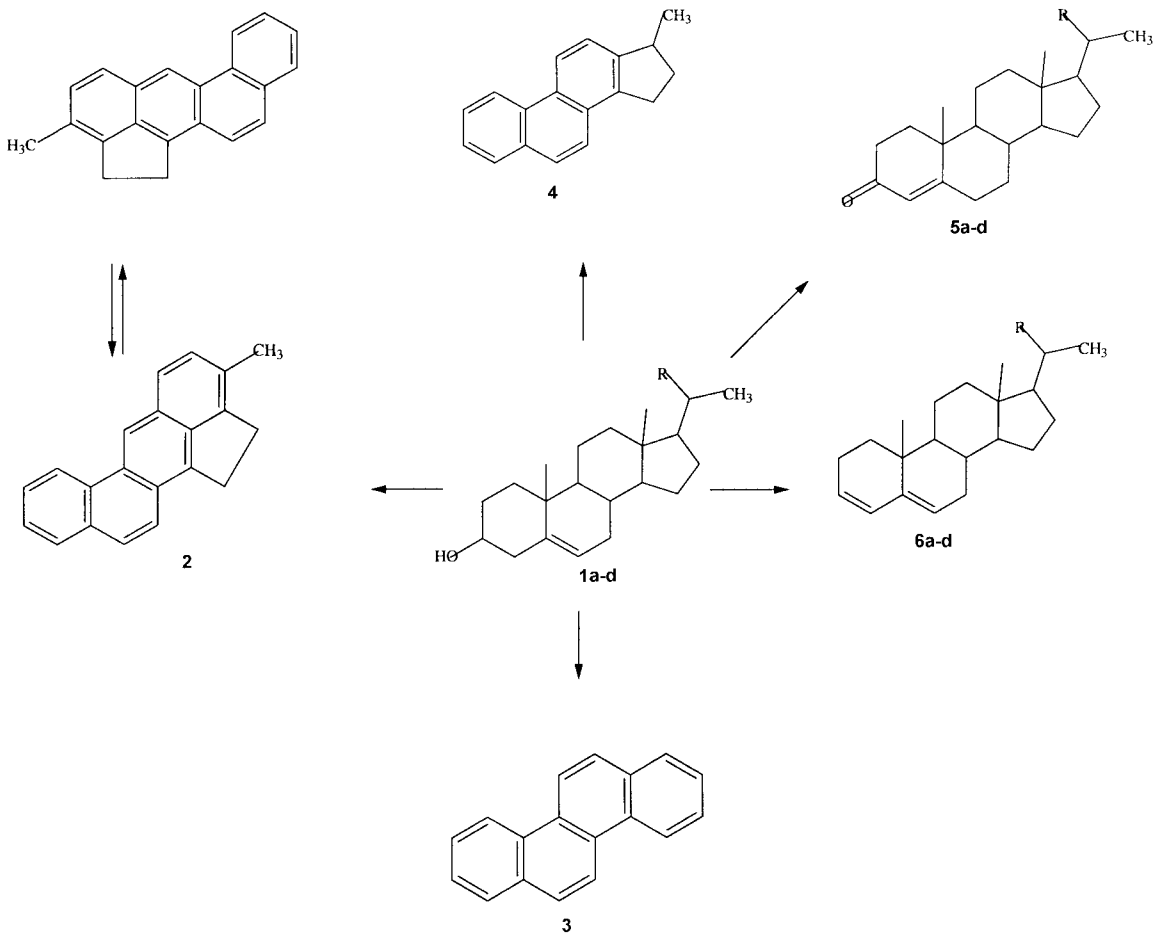

Figure 1. Possible sterol degradation products
1a = cholesterol, R = -$(CH_2)_3$-$CH(CH_3)_2$; **1b** = campesterol, R = -$(CH_2)_2$-$CH(CH_3)$-$CH(CH_3)_2$; **1c** = β-sitosterol, R = -$(CH_2)_2$-$CH(C_2H_5)$-$CH(CH_3)_2$; **1d** = stigmasterol, R = -CH=CH-$CH(C_2H_5)$-$CH(CH_3)_2$;
2 = 1,2-dihydro-3-methylbenz[*j*]aceanthrylene (3-methylcholanthrene); **3** = chrysene; **4** = Diels' hydrocarbon;
5a = 4-cholesten-3-one; **5b** = 4-campesten-3-one; **5c** = β-4-sitosten-3-one, **5d** = 4-stigmasten-3-one;
6a = 3,5-cholestadiene; **6b** = 3,5-campestadiene; **6c** = β-3,5-sitostadiene; **6d** = 3,5-stigmastadiene

were found and identified (48). The homolog, tetraisovalerylglucose[2], is no longer used in smoking products, even though Ames testing (*Salmonella typhimurium*) demonstrated it to be nonmutagenic.

Radiotracer studies on the transfer and fate of the additive phenylethanol showed that a substantial portion of it was converted to benzoic and phenylacetic acids during the smoking process (49).

In their 1989 publication on the results of their study of six model flavorants added to cigarettes and their distribution among the cigarette smoke fractions, GREEN *et al.* (BAT-UK) commented on the paucity of publications on the relationship between flavorant additions and smoke composition (50):

> Apart from menthol, which has been studied in detail by Jenkins *et al*. (14) [*sic*[3]], and vanillin, recently examined by Green *et al*. (51) [RJRT], little is published regarding the extent of pyrolytic decomposition of the chemicals involved.

The six model compounds studied by GREEN *et al.* (50) are highly flavorful. They comprised radiolabeled anisaldehyde (4-methoxybenzaldehyde), anisole (methoxybenzene), benzaldehyde, isoamyl isovalerate, methyl cinnamate, and vanillin (4-hydroxy-3-methoxybenzaldehyde). Only one of the six, anisaldehyde, showed any significant decomposition during the smoking process. The findings by GREEN *et al.* (BAT-UK) with respect to vanillin were in agreement with those reported previously by GREEN *et al.* (RJRT) (54). In the BAT study, it was found that several of the model flavorants, e.g., benzaldehyde and isoamyl isovalerate, were rapidly lost from the flavorant-treated cigarettes during an 8-week equilibration period.

[2]Isovaleric acid, which is not optically active, is a commercially available compound. 3-Methylvaleric acid in the naturally occurring sugar ester is optically active and is present as the *d* or (+) isomer. To synthesize and separate the required optically active isomer in the quantities needed for large-scale production of the natural occurring acetyl-*tris*-(3-methylvaleryl)glucopyranoside is extremely expensive.

[3]It should be noted that GREEN *et al.* (BAT-UK) neglected to cite several published RJRT studies: The 1968 report by NEWELL *et al.* (52) on menthol, the 1984 and 1986 reports by GREEN *et al.* (53) on phenylacetic acid, or the 1987 and 1988 reports by LYNM (49) on phenylethanol.

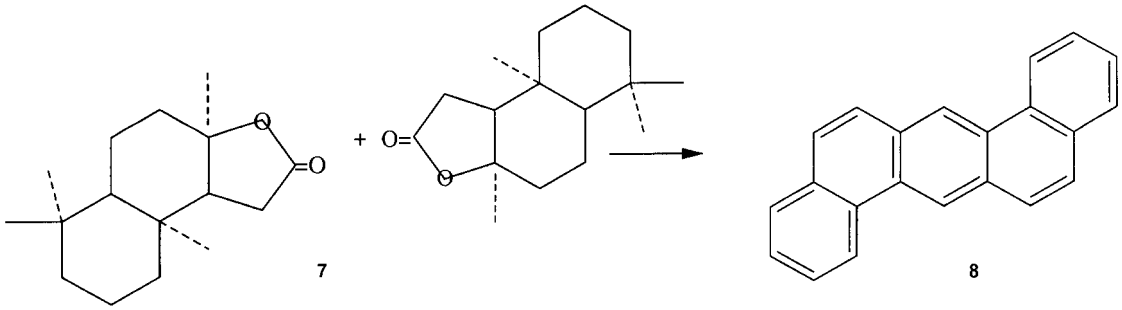

Figure 2. Theoretical conversion of sclareolide (decahydro-2-hydroxy-2,5,5,8a-tetramethyl-1-naphthaleneacetic acid, lactone) (7) to dibenz[*a,h*]anthracene (DBA) (8)

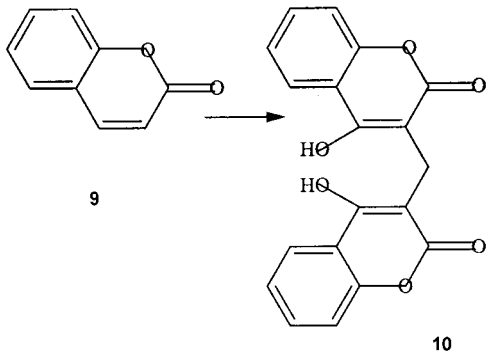

Figure 3. Theoretical conversion of coumarin (2*h*-1-benzopyran-2-one) (9) to dicumarol (3,3'-methylene*bis*[4-hydroxy-2*H*-1-benzopyran-2-one]) (10)

Much research had been conducted since the mid-1950s to define the precursors in tobacco of allegedly harmful components such as the PAHs, the phenols, the aldehydes and ketones, the aza-arenes, etc. in tobacco smoke, particularly cigarette MSS. As noted, these major precursors – in contrast to compounds or materials used as flavorants or top dressing components – are generally characterized by two attributes (cf. Table 3): Relatively high molecular weights (molecular weights substantially greater than 200 to 250) and low volatility. In general, past studies on these precursors, particularly those that generate relatively high levels of PAHs, have involved a) their almost complete removal from tobacco by solvent extraction which was usually followed by a decrease in PAH levels in the MSS from a cigarette fabricated with the extracted tobacco or b) their addition to tobacco in quantities approximating those already present in the tobacco which was followed by an increase in the PAH levels in the treated-tobacco MSS (37), i.e., *milligram* quantities were added in the cases involving the saturated aliphatic hydrocarbons, solanesol, and β-sitosterol (see Table 4).

Some concern has been expressed recently that very little of the in-house (or outside) research since the mid-1950s has dealt with the contributions of flavorants, added at levels of a few micrograms per gram of tobacco blend, to the levels of allegedly harmful components in cigarette MSS. Some 30 examples of such investigations are summarized in Table 5.

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is a flavorful compound used for many years not only as a tobacco flavorant but also as a flavorant in beverages, foodstuffs, and confections. To resolve an issue whether vanillin was converted to phenol during the smoking process as claimed by KATO and SHIBAYAMA (55), EBLE *et al*. (56) determined the fate of ^{14}C-radiolabeled vanillin in a cigarette during smoking. No ^{14}C-radiolabeled phenol was found in the MSS, indicating none was formed from the ^{14}C-radiolabeled vanillin, a result contrary to that reported earlier by KATO and SHIBAYAMA (55). Of the ^{14}C-radiolabeled material in the phenols-rich fraction of the MSS, 99.8% was unchanged ^{14}C-radiolabeled vanillin. The difference in results are readily explained when the experimental conditions are examined: In their study (*substantial vanillin-to-phenol conversion*), KATO and SHIBAYAMA used *continuous* draw in their smoking regime, i.e., no alternating puff and smolder period, whereas EBLE *et al.* in their study (*no vanillin-to-phenol conversion*) used the intermittent-puff smoking regime (35-mL puff volume, 2-sec puff duration, 1 puff/min; 25 ˚C, 60% RH, etc.) defined by the FTC in the USA and by the Cooperation Center for Scientific Research Relative to Tobacco (CORESTA) in Europe for smoking cigarettes whose MSS was to be analyzed.

In 1967, WYNDER and HOFFMANN, after summarizing several studies to that date on the effect of humectants (glycerol, propylene glycol) and casing materials (sugars, licorice) on cigarette smoke composition (57), commented as follows on the need for further research on the effect of tobacco flavor-enhancing additives on MSS composition and properties:

> However, the use of such agents [that enhance tobacco flavor] may mean that precursors of irritants or toxic substances in the smoke are introduced. The pyrolysis products or the smoke of cigarettes with flavor additives were rarely tested in biologically assays. This area of research deserves intensified investigation.

Since the publication of the 1967 WYNDER-HOFFMANN book, considerable research has been conducted on the effect of casing materials (sugars, cocoa) and humectants on cigarette smoke properties. Much of this research is summarized elsewhere (18). The claim by many investigators opposed to tobacco smoking that a) lack of knowledge of the nature of the proprietary materials used as tobacco

Table 5. Flavorants and other components examined individually for their effect on cigarette mainstream smoke composition

Additive	[mol. wt.]	Table[a]	Reference(s)
p-Anisaldehyde [benzaldehyde, 4-methoxy-]	[136]	IV	STOTESBURY *et al.* (33,34); GREEN *et al.* (50)
Anisole [benzene, methoxy-]	[108]	VIII	STOTESBURY *et al.* (33,34); GREEN *et al.* (50)
Benzaldehyde	[106]	IV	STOTESBURY *et al.* (33,34); GREEN *et al.* (50)
sec-Butylmalonic acid[b]	[160]	I	RODGMAN and COOK (37)
Citric acid	[192]	I	BEST (92,93); NEWELL and BEST (94)
Coumarin [2*H*-1-benzopyran-2-one]	[146]	XI	NEWELL (44)
Cystine[c]	[240]	II	RODGMAN and COOK (37)
Glycerol	[92]	III	BEST (92,95,96); BEST *et al.* (97); BEST and FRIENDE (98)
Guaiacol [phenol, 2-methoxy-]	[124]	XII	BEST (92)
cis-3-Hexen-1-ol[d]	[100]	III	BEST (92); BEST and SINK (99)
Isoamyl isovalerate	[172]	VII	STOTESBURY *et al.* (33,34); GREEN *et al.* (50)
Isopropylmalonic acid[b]	[146]	II	RODGMAN and COOK (37)
Levulinic acid [pentanoic acid, 4-oxo-]	[118]	I	EBLE (100); BEST (101)
Linalool	[154]	III	BEST (92); BEST and SINK (102)
l-Malic acid	[134]	I	NEWELL and BEST (103)
Menthol[e]	[156]	III	STOTESBURY *et al.* (34); BEST (92,104); EBLE and SINK (105); EBLE *et al.* (106); BOCK *et al.* (16); JENKINS *et al.* (14); NEWELL (107); NEWELL and LATIMER (108); NEWELL *et al.* (109)
Methionine[c]	[149]	II	RODGMAN and COOK (37)
Methyl cinnamate [2-propenoic acid, 3-phenyl-, methyl ester]	[162]	VII	STOTESBURY *et al.* (33,34); GREEN *et al.* (50)
Nicotine	[162]	—	BEST (96,110,111); SCHMELTZ *et al.* (112)
Nicotine levulinate	[278]	—	BEST (110)
1-Octen-3-ol	[128]	III	BEST (92)
Oxalic acid	[90]		NEWELL and BEST (113)
Phenylacetic acid	[136]	I	BEST (92); GREEN *et al.* (54); MORRISON *et al.* (114,115); NEWELL (116)
Phenylethanol	[122]	III	BEST (92); LYNM (49)
Phenethyl isovalerate	[206]	VII	BEST (92); BEST and SINK (117)
Propane-1,2-diol	[76]	III	BEST (92); BEST *et al.* (118)
Sclareolide[f]	[250]	XI	NEWELL (119); RODGMAN and COOK (37)
α-Terpineol	[154]	III	BEST (92)
α-Terpineol acetate	[196]	VII	BEST (92)
Trimyristin	[722]	—	RODGMAN and COOK (37)
Vanillin [benzaldehyde, 4-hydroxy-3-methoxy-]	[152]	IV	STOTESBURY *et al.* (33,34); BEST (92); BEST *et al.* (120,121,122); EBLE *et al.* (56); GREEN *et al.* [RJRT (53)]; GREEN *et al.* [BAT (50)]; KATO and SHIBAYAMA (55); MORRISON and BEST (123)

[a]Tables cited are those of LEFFINGWELL *et al*. (19).

[b]The added malonates generate methylbutyric and methylvaleric acids during smoking. These acids are characteristic components of the MSS from Oriental tobaccos. The malonates were actually added at levels of mg/g of tobacco in the smoke study.

[c]In the original study, this sulfur-containing amino acid was not assessed as a flavorant *per se*, but an attempt was made to determine whether, by generating elemental sulfur during the smoking, it would enhance the pyrogenesis of PAHs. Saturated cyclic/polycyclic compounds when heated with sulfur (or selenium) yield cyclic aromatic hydrocarbons and PAHs. For example, phytosterols, which usually are saturated tetracyclic structures, yield a cyclopentaphenanthrene (Diels' hydrocarbon) when heated with sulfur (or selenium).

[d]Also known as leaf alcohol.

[e]The menthol level in some commercial cigarettes may approach 8 mg/g of tobacco, so menthol is generally not considered as a flavorant added at "microgram levels".

[f]Sclareolide was added at a level of 4.5 mg/g of tobacco in the smoke study.

flavorants or in "top dressing" formulations and b) their use levels precluded meaningful research on their effect on cigarette smoke composition and properties was offset in 1972 with the publication by RJRT of a monograph by LEFFINGWELL *et al*. (17) on such flavorful materials, either actually used to flavor tobacco smoking products or proposed in numerous patents for such use. Despite the publication of this monograph, in which nearly a thousand materials – either used or proposed for use as flavorants for tobacco smoking products – were described and listed according to chemical class (acids, alcohols, esters, etc.), very little research on the effect of any of the tobacco flavorants listed has been reported from the laboratories of the investigators questioning the use of these flavorants. The bulk of the research on the effect of such tobacco flavorants on the composition and properties of cigarette MSS has been reported from the research laboratories of the Tobacco Industry members.

The flavorant issue raises several questions about their use and their contribution to cigarette smoke composition. For instance, have the knowledge and understanding gained over the past two decades (see reviews by BAKER [58] and publications cited therein) on cigarette MSS formation and transport through the cigarette tobacco rod made it obvious to all knowledgeable researchers that the behavior of a highly volatile, generally low molecular weight flavorant (see Tables 3 and 5) in a burning and puffed cigarette differs substantially from that of a relatively nonvolatile,

Table 6. Experiment design: Flavorants, casing materials, and humectants

Cigarette[a] variation	Flavorant formulation level	Casing[b] and humectants[c] 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

[a]Cigarette brands included Winston KS, Salem KS, Vantage KS, Camel Filter KS, and Now KS manufactured in early 1977.
[b]Licorice, cocoa, and sugars.
[c]Glycerol and propylene glycol.

high molecular weight precursor (see Tables 3, 4, and 5) of allegedly harmful smoke components? This point has been discussed at length previously in this memorandum. Is this the major reason why so little effort on the contribution of added flavorants to MSS composition and properties has been expended by investigators both within and outside of the Tobacco Industry? As indicated by the references cited for the compounds listed in Table 5, the bulk of the published work has dealt with the efficiency of transfer of specific flavorants from the tobacco rod to the MSS (and thus to the smoker).

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 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.

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*., 31).

As an alternate to this arduous, expensive, and almost insurmountable task of studying individually the effect of several hundred 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 flavor formulations were qualitatively and quantitatively unique for each commercial brand and 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

Table 7. Summary of mutagenicity data from various cigarette smoke condensates (59)

RJRT Brand	Strain	Mutagenicity in revertant/plate[a] A[b]	B	C	D
Winston KS	TA1538	200[c]	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

[a]For CSC at 500 μg/plate with *Salmonella typhimurium* for cigarette variation.
[b]See Table 6 for description of cigarette variations.
[c]Each value is the average of 10 determinations.

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 6.

The MSS total particulate matter (TPM) from each of these four cigarette variations for five RJRT brands (Winston KS, Salem KS, Vantage KS, Camel Filter KS, and Now KS) was examined for 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 obtained (Table 7), it was concluded (59):

> 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 wet total particulate matter (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 (59).

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.

Inclusion of humectants (glycerol, propylene glycol, and/or triethylene glycol) in the tobacco blend results in transfer of substantial amounts of them from the tobacco rod to both the MSS and SSS: HEGE (60) and SWICEGOOD (61,62) reported that the MSS FTC "tar" from numerous commercial cigarettes contained significant percentages of humectants. These data are included in the companion article (18).

Inclusion of glycerol and/or propylene glycol in the cigarette tobacco blend results in their transfer in significant amounts from the tobacco filler to the MSS where they are found primarily in the TPM (63). Thus, it is not surprising that their removal from the additive system produces TPM with increased mutagenicity (59). The two compounds, both nonmutagenic, act as diluents for the remainder of the MSS TPM components produced pyrogenetically or transferred directly from the tobacco rod to the smoke during the smoking process.

From these data (Table 7), it is apparent that the flavorant formulation used in the commercial brands studied does not increase its MSS specific mutagenicity. In fact, the flavorant 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 for five different commercial products, do not contribute components to the smoke whose levels and/potency are such that they produce abnormal increases in the specific mutagenicity as measured in the Ames *Salmonella typhimurium* test system.

To the knowledge gained in the 1950s on the effect of compounds identical with or similar to those used in cigarette flavor formulations on the chemical composition of MSS, particularly its PAH content (35), and in the 1970s on the effect of product flavor formulations on MSS specific mutagenicity as measured in the Ames *Salmonella typhimurium* test was recently added definitive knowledge on the effect of addition of a mixture of selected flavor formulation components to cigarette tobacco on laboratory animals a) exposed to the resulting MSS by inhalation and b) treated via skin painting with the resulting CSC.

Among the flavorants added to the tobacco blend, menthol is a special case since, as noted previously, its addition level is several magnitudes greater than that of any of the other components of the flavor formulation. Chemically, its fate during smoking was well defined by the results reported in 1968 by NEWELL *et al*. (64) and in 1970 by JENKINS *et al.* (14) from their studies with ^{14}C-menthol added to tobacco. The level of menthol in the MSS, SSS, and the butt indicated that less than 2% of the added menthol underwent pyrolysis during the smoking process. Biologically, the added menthol produces little change in the effects studied. a) As noted previously, in 1965 BOCK *et al*. (16) reported no difference between the specific tumorigenicities of the CSCs from non-mentholated vs. mentholated cigarettes. b) Increasing the levels of the flavorant formulation and the menthol on the tobacco blend of the Salem KS cigarette by a factor of 10 produced no significant change in specific mutagenicity in the Ames test (Tables 12 and 13 in [59]). c) In a 13-week inhalation study with rats, GAWORSKI *et al.* (65) reported in 1997 that addition of 5000 ppm of menthol to the tobacco blend had no substantial effect on the character or extent of the biological responses normally associated with inhalation of cigarette MSS.

Almost 77% of the items listed by DOULL *et al.* (1) as ingredients added by the six major US cigarette manufacturers during cigarette manufacture are individual compounds, the remaining items are mixtures, e.g., natural oils, plant extracts, oleoresins. Many of the individual compounds fell into one of the following categories: a) It was a component of one or more of the tobacco types (flue-cured [66], burley, Oriental, Maryland) commonly used in cigarette blends. b) It was a component of cigarette MSS (63). c) It was a component of both tobacco and tobacco smoke. d) It was an homolog or isomer of an identified tobacco and/or tobacco smoke component.

In their study of the effect of added ingredients on the biological effect of inhaled cigarette MSS, GAWORSKI *et al*. (67) administered to rats via inhalation the MSS from cigarettes to which 172 ingredients (129 individual compounds, 43 mixtures) had been added. Most of the ingredients were included in the DOULL *et al.* list. From the results of their inhalation experiment, GAWORSKI *et al*. concluded:

> the addition of these flavoring ingredients to cigarette tobacco had no discernible effect on the character or extent of the biological responses normally associated with inhalation of mainstream cigarette smoke in rats.

In a similar biological study, GAWORSKI *et al*. (68) investigated the effect on the specific tumorigenicity of the CSC from cigarettes to which 150 ingredients (109 individual compounds, 41 mixtures) had been added. Here again, most of the ingredients were included in the DOULL *et al.* list. From the results of their mouse skin-painting experiment, GAWORSKI *et al*. concluded:

> While tumor incidence, latency and multiplicity data occasionally differed between test and comparative reference CSC groups, all effects appeared to be within normal variation for the model system. Furthermore, none of the changes appeared to be substantial enough to conclude that the tumor promotion capacity of CSC obtained from cigarettes containing tobacco with ingredients was discernibly different from the CSC obtained from reference cigarettes containing tobacco processed without ingredients.

Much has been asserted recently about the effect of ammoniation of tobacco on MSS pH, MSS nicotine delivery, the form of the MSS nicotine, the perception of the nicotine by the smoker, and the smoker's supposed acceptance of these MSS changes. Little attention has been paid to the effect of the ammoniation on other aspects of the MSS composition, its perception by the consumer, and the smoker's acceptance of these changes. Usually the pH of the MSS from ammoniated tobacco is increased slightly over that from the control tobacco, but other MSS compositional changes are much more significant. For example, the MSS from a cigarette containing ammoniated tobacco a) shows an increased per cigarette level of ammonia, b) frequently shows a decreased per cigarette delivery of nicotine because the nicotine content of its filler is lowered by some types of ammoniation (69), c) shows decreased levels of MSS vapor-phase components considered irritants (formic and acetic acids; formaldehyde, acetaldehyde, acrolein [69]; acetone), d) shows modest increased levels of alkylpyridines classified as contributors to the harshness and undesirable taste of MSS (70), and (e) shows substantially per cigarette increased MSS deliveries of alkylpyrazines (71), many of which are highly flavorful and known components not only of cigarette MSS[4] but also of

[4]Pyrazine plus over 50 alkylpyrazines have been identified in tobacco smoke.

heated foodstuffs and beverage sources, e.g., coffee, tea, cocoa, peanuts, roast meats (71,72). Cigarette MSS flavor and aroma may be enhanced by addition of appropriate alkylpyrazines to the tobacco blend, e.g., of the 460 pure compounds listed as possible cigarette tobacco ingredients by DOULL *et al*. (1), 23 (5%) are pyrazine derivatives. The effects of various ammoniation treatments of tobacco on cigarette MSS composition were recently summarized (73). Thus, the consumer acceptance of the MSS from an ammoniated tobacco cigarette may be more logically attributed to its increased mildness (decreased irritancy from lower per cigarette deliveries of low molecular acids and carbonyl compounds) and enhanced flavor and aroma (increased per cigarette deliveries of flavorful alkylpyrazines) rather than to its slightly increased pH and the supposed effect of increased MSS pH on enhanced nicotine delivery and modified nicotine properties (73).

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.

From their recent detailed assessment of the chemical and biological properties reported in the published literature for the MSSs from cigarettes fabricated with tobacco with or without one or more additives, PASCHKE *et al*. (2) concluded:

> In general, no significant increase in the biological activity (first of all carcinogenicity, mutagenicity and cytotoxicity) of tobacco was reported from cigarettes containing ingredients.

A detailed critique of the information available on the additives used primarily in flavor formulations reinforces the conclusions presented in the reports by DOULL *et al*. and PASCHKE *et al*. Even though the flavor formulation or "top dressing" for the blend in a specific cigarette brand may comprise as many as 100 individual components, their total weight seldom exceeds 2 mg/g of tobacco blend, i.e., the individual components are added at the μg/g level.

A similar critique of the components of the casing material and humectant formulations appears in a companion report (18). Compared to the levels used for several of the individual casing material ingredients, the flavorant mixture represents a minor addition.

Inclusion of modest levels of the flavor formulations 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. A ten-fold increase in the flavorant formulation addition produced no significant change in the MSS mutagenicity as measured by the Ames test *Salmonella typhimurium* bioassay.

Results previously unpublished but currently available in various Federal and State repositories are presented to indicate that several of the flavor formulation components which theoretically could generate undesirable components during the smoking process do not do so. For example, the Oriental tobacco component sclareolide could theoretically generate DBA (Figure 2). However, tobacco "spiked" with many times the proposed use level of sclareolide showed no increased MSS level of DBA. Coumarin, a flavorant no longer used in the US Tobacco Industry, could theoretically yield dicumarol, a potent anticoagulant (Figure 3). However, no radiolabeled dicumarol was detected in the MSS from radiolabeled coumarin-treated tobacco cigarettes. The failure to detect radiolabeled phenol in the MSS from radiolabeled vanillin-treated tobacco cigarettes disproved the assertion that vanillin did not transfer *per se* to MSS but was primarily converted to phenol during smoking.

On the basis of this critique, it is concluded that the components of the flavorant formulation ("top dressing") added to tobacco in the manufacture of cigarettes are not hazardous under the conditions of use.

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NOTE ADDED IN PROOF

Near the end of the most recent procedure to obtain approval for publication of the preceding manuscript in *Beiträge zur Tabakforschung International/Contributions to Tobacco Research*, the author obtained copies of four publications by CARMINES and his colleagues (124–127) on their excellent study of the effects of ingredients added to a cigarette on the chemical and biological properties of its MSS.

A total of 333 ingredients commonly used in cigarette manufacture was added to a typical commercial blended cigarette. Ingredients were added at approximately the levels normally used in commercial cigarettes and at levels several times those normally used. The MSS data vs. those from a control cigarette with no added ingredients indicated

an increase in the TPM. Normalizing the yields of individual MSS ingredients to the TPM yields indicated a reduction in the majority of them. An increase in the amount relative to TPM was observed for only a few MSS components (125). These chemical results on the MSSs were consistent with the results obtained not only in *in vitro* mutagenicity and cytotoxicity studies with the TPMs from the ingredient-treated and control cigarettes (126) but also in *in vivo* studies with rats exposed via inhalation to the MSSs from the treated and control cigarettes (127): The addition of the ingredients did not increase the mutagenicity or cytotoxicity of the TPMs from the ingredient-treated cigarettes or the inhalation toxicity to rats of their MSSs.

These findings not only bolster the observations reported in the present and a companion manuscript (18) but also the conclusions reached by DOULL *et al*. (1), PASCHKE *et al*. (2), and GAWORSKI *et al*. (65,67,68) on the effect of added ingredients from those listed by DOULL *et al*. (1).

Over the years it has been repeatedly asserted that cigarette ingredients added at normal levels to pre-1980 cigarettes or at slightly increased levels to more recent lower "tar" cigarettes might adversely modify the chemistry and biology of the MSSs from such cigarettes. However, no chemical or biological evidence has been presented in support of such assertions.

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