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Studies of Polycyclic Aromatic Hydrocarbons in Cigarette Mainstream Smoke: Identification, Tobacco Precursors, Control of Levels: A Review*

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SUMMARY

During the period of tobacco smoke research from the early 1950s to the mid-1960s it was repeatedly asserted that a) tobacco and many tobacco components were involved in the pyrogenesis of polycyclic aromatic hydrocarbons (PAHs), several of which were reported to initiate tumors on the skin of laboratory animals and b) tobacco additives (flavorants, casing materials, humectants) were highly likely to be similarly involved in PAH pyrogenesis.
Extensive knowledge on PAHs was deemed highly necessary because of their claimed importance in the smoking–health issue. The numerous assertions about the generation of PAHs in cigarette mainstream smoke (MSS) triggered extensive and intensive research both within and outside the Tobacco Industry to define the nature of the PAHs, their per cigarette MSS delivery amounts, their precursors, etc.
It was not until 1960 that VAN DUUREN *et al*. (1) reported three specific aza-arenes in cigarette MSS that were asserted to be involved in smokers' respiratory tract cancer. As noted in a recent Letter to the Editor (2), the presence of these three aza-arenes in tobacco smoke has never been confirmed. Between 1960 and 1965, other MSS components (phenols as promoters, polonium-210, *N*-nitrosamines, ciliastatic compounds) were asserted to be responsible for smoking-related diseases. However, no major assertions were made that phenols, polonium-210, or the *N*-nitrosamines were derived from flavorants, casing materials, or humectants. Some investigators did report that several ciliastats were derived from added sugars and glycerol. The ciliastat proposal was drastically diminished in importance by the findings in the 1960s that only a relatively small proportion of the ciliastats reached the smoker's cilia.
During that time, pertinent skills and competencies in research on tobacco smoke composition, particularly the PAH fraction, have been developed. Such skills permitted the isolation in crystalline form of 14 PAHs and the quantitation of these and many other PAHs. They were also used to put in perspective the pyrogenesis of PAHs from a) specific tobacco components, b) additives, and c) processed tobaccos (reconstituted tobacco sheet [RTS], expanded tobacco). R.J. Reynolds Tobacco Company (RJRT) pioneered the use of RTS (1953) and expanded tobaccos (1969) in cigarette blends and generated much previously unpublished data on the effect of such processed tobaccos on MSS composition. [Beitr. Tabakforsch. Int. 19 (2001) 361–379]

ZUSAMMENFASSUNG

Während der Periode der Tabakforschung von Beginn der 50er bis zur Mitte der 60er Jahre wurde wiederholt behauptet, dass a) Tabak und viele Tabakinhaltshaltsstoffe

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an der Entstehung polycyclischer aromatischer Kohlenwasserstoffe (PAH) beteiligt seien, wovon einige, wie in Untersuchungen berichtet, bei Versuchstieren Tumoren auf der Haut hervorrufen und b) Tabakadditive (Aromatisierungsmittel, Sossierungsmaterial, Feuchthaltemittel) mit großer Wahrscheinlichkeit ebenfalls an der Pyrosynthese der PAHs beteiligt seien.
Aufgrund ihrer damaligen Bedeutung für das Thema Rauchen und Gesundheit wurde ein umfassendes Wissen über PAHs als äußerst wichtig erachtet. Die Vielzahl an Behauptungen über die Entstehung der PAHs im Hauptstromrauch (HSR) von Cigaretten führte zu umfassenden und intensiven Forschungstätigkeiten innerhalb und außerhalb der Tabakindustrie, um die Natur der PAHs, ihre mengenmäßige Freisetzung im HSR von Cigaretten, ihre Vorläufersubstanzen usw. zu bestimmen.
Erst im Jahre 1960 haben VAN DUUREN *et al.* (1) über drei spezifische Azaarene im HSR von Cigaretten berichtet, die bei Rauchern an der Krebsentstehung der Atemwege beteiligt sein sollten. Aus einem kürzlich erschienenen Brief an die Herausgeber (2) geht hervor, dass die Existenz dieser Azaarene im HSR von Cigaretten niemals bestätigt wurde. Zwischen 1960 und 1965 wurde von anderen Inhaltsstoffen des HSRs (Phenole als Promotoren, Polonium-210, *N*-Nitrosamine, ciliastatische Verbindungen) behauptet, sie seien für die Gesundheitsschäden durch Rauchen von Bedeutung. Es gab jedoch keine grundlegenden Berichte, dass Phenole, Polonium-210 oder *N*-Nitrosamine aus Aromasubstanzen, Sossierungsmaterial oder Feuchthaltemitteln entstanden seien, wohingegen es jedoch einige Untersuchungen gab, denen zufolge mehrere ciliastatische Verbindungen aus zugesetztem Zucker und Glycerol entstanden seien. Dieser ciliastatische Ansatz verlor durch neue Erkenntnisse in den 60er Jahren stark an Bedeutung, wonach nur ein relativ geringer Anteil der ciliastatischen Substanzen die Flimmerhärchen der Raucher erreichte.
Während dieser Zeit wurden einschlägige Sachkenntnisse und Fähigkeiten auf dem Gebiet der Tabakrauchinhaltsstoffe, insbesondere im Bereich der PAHs, erworben. Eine derartige Sachkenntnis erlaubte die Isolierung von 14 PAHs in kristalliner Form sowie die Quantifizierung dieser Substanzen und vieler anderer PAHs. Mithilfe dieser speziellen Sachkenntnis wurde auch die Bedeutung der Entstehung der PAHs aus a) spezifischen Tabakinhaltsstoffen, b) Additiven und c) verarbeitetem Tabak (Tabakfolie [Reconstituted Tobacco Sheet = RTS], Blähtabak) näher untersucht. R.J. Reynolds Tobacco Company (RJRT) verwendete als erstes Unternehmen RTS (1953) und Blähtabak (1969) in Tabakmischungen und ermittelte zahlreiche bislang unveröffentlichte Daten über die Auswirkungen solcher verarbeiteten Tabake auf die Zusammensetzung des HSR. [Beitr. Tabakforsch. Int. 19 (2001) 361–379]

RESUME

Du début des années 1950 jusqu'au milieu des années 1960 il a été prétendu maintes fois que a) le tabac et plusieurs composants du tabac contribuent à la pyrogénèse des hydrocarbures polynucléaires aromatiques (PAH), dont il a été rapporté qu'ils causent des tumeurs sur la peau d'animaux de laboratoire, et b) que les additifs du tabac (aromatisants, produits de sauçage, humectants) contribuent vraisemblablement également d'une façon comparable à la pyrogénèse des PAHs.
Les connaissances approfondies sur les PAHs étaient considérées comme nécessaires à cause de leur importance prétendue dans la controverse tabac–santé. Les nombreuses allégations sur la formation des PAHs dans le courant principal (CP) de la fumée des cigarettes ont conduit à des recherches extensives et intensives à l'intérieur et à l'extérieur de l'industrie du tabac en vue de déterminer la nature des PAHs, leur rendement dans le CP des cigarettes, leurs précurseurs, etc.
En 1960 seulement, VAN DUUREN (1) *et al.* ont communiqué l'existence de trois aza-arènes dans le CP des cigarettes, et dont il a été avancé qu'ils contribuent à la formation du cancer du système respiratoire chez les fumeurs. Comme il était rapporté dans une lettre aux éditeurs (2), l'existence de ces trois aza-arènes dans la fumée du tabac n'a jamais été prouvée. Entre 1960 et 1965, d'autres composés (phénols comme promoteurs, polonium-210, *N*-nitrosamines, composés «ciliastatiques») étaient considérés comme responsable pour les maladies liées au fumage. Cependant, il n'y avait pas d'affirmation significatives que ces composés étaient formés à partir d'aromatisants, des produits de sauçage ou des humectants, bien qu'il y ait quelques études selon lesquelles les composés «ciliastatiques» étaient formés à partir de sucres et de glycérol apportés au tabac. L'approche «ciliastatique» a perdu de son importance par les résultats obtenus au cous des années 1960, que seules des quantités relativement faibles en composés «ciliastatiques» atteignent les cils des fumeurs.
A cette époque, des connaissances particulières et des compétences dans la recherche sur la composition du tabac, et surtout dans le domaine des PAHs, ont été obtenues. De telles connaissances ont permit d'isoler 14 PAHs cristallins et de les doser, ainsi que beaucoup d'autres PAHs. Grâce à ces connaissances, on a pu examiner l'importance de la pyrogénèse des PAHs à partir a) des composants spécifiques du tabac, b) des additifs et c) du tabac manufacturé (tabac reconstitué, tabac expansé). R.J. Reynolds Tobacco Company (RJRT) a été le premier à utiliser du tabac reconstitué (1953) et du tabac expansé (1969) dans les mélanges pour cigarettes et a fournit auparavant des données non publiées sur l'effet du tabac manufacturé sur la composition du CP. [Beitr. Tabakforsch. Int. 19 (2001) 361–379]

I. INTRODUCTION AND BACKGROUND

Between 1950 and 1970, a wealth of information on tobacco-related topics was generated and several significant cigarette design technologies were developed to

modify the delivery and composition of cigarette mainstream smoke (MSS). These activities were triggered by a) results in the early 1950s from retrospective epidemiology studies (3) in which it was reported that an association existed between cigarette smoking and the incidence of lung cancer in smokers, and b) the 1953 report of the production of skin carcinoma in susceptible laboratory animals skin painted repeatedly with a concentrated solution of cigarette MSS condensate supposedly produced under conditions simulating the human smoking of a cigarette (4).

These reported findings raised several questions, and both the questions and their answers led to several controversies. The first question posed dealt with the identity of the cigarette MSS component(s) responsible for the smoking–lung cancer association and the skin tumor induction in laboratory animals. Because of extensive data generated on the specific tumorigenicity of about 25% of the hundreds of polycyclic aromatic hydrocarbons (PAH) synthesized between 1929 and the early 1950s (5), PAHs were immediately indicted as the responsible agents in cigarette MSS even though their presence was not certain. Eventually, numerous PAHs were identified in cigarette MSS. Because of its MSS level and its high specific tumorigenicity in several bioassays, one PAH was subjected to intense scrutiny: Benzo[*a*]pyrene (B[*a*]P). As a carcinogen, B[*a*]P elicited carcinomas at the painting site in the mouse-skin bioassay. As a sarcogen, B[*a*]P elicited sarcomas in rodent bioassays involving subcutaneous injection.

The controversy on the questions of the presence of PAHs in general and B[*a*]P in particular in cigarette MSS began in the early 1950s. Despite several British and American reports in 1954 and 1955 of its identification in cigarette MSS, FIESER (6) concluded from the data published prior to his 1957 publication that the presence of B[*a*]P in MSS had not yet been adequately demonstrated. However, before 1960, both questions were resolved: Numerous PAHs, including B[*a*]P, were unequivocally identified as MSS components.

Once the presence of PAHs in MSS was established, the nature of their major precursors in tobacco was sought. Initially, numerous invalid assertions were made about the source of PAHs in MSS but eventually their major precursors were defined as several of the lipophilic classes of components in tobacco, e.g., the phytosterols, terpenoids such as solanesol, and the saturated aliphatic hydrocarbons. Actually, the controversy on the presence of PAHs in cigarette MSS was unnecessary because it had long been known that a) PAHs could be pyrosynthesized from any organic compound with a C-H linkage (7), b) tobacco comprised numerous organic compounds with the necessary C-H linkage, and c) the tobacco smoking process provided the heat required to generate PAHs.

The numerous assertions (many of them shown later to be incorrect [8]) on the presence or absence of PAHs in cigarette MSS, their precursors in tobacco, their mechanism of formation, their contribution to laboratory animal tumorigenesis, and their possible involvement in the smoking–health issue led to extensive research on ways to remove them from or to reduce their levels in MSS. Obviously, a successful solution might have had considerable proprietary value. Beginning in late 1954, such research was initiated at R.J. Reynolds Tobacco Company (RJRT) R&D. The first task was to resolve the question whether PAHs were indeed present in MSS, which ones, and their per cigarette MSS delivery levels. If they were present, the next task was to develop a means to reduce their level in MSS, particularly the levels of those PAHs reported to be tumorigenic in various laboratory bioassays.

II. IDENTIFICATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN CIGARETTE MAINSTREAM SMOKE

In 1954, knowledge of cigarette MSS composition was extremely limited. KOSAK (9) listed fewer than 100 components reported in tobacco smoke and many of those listed were incorrect. As a prelude to discussion of the effect of added flavorants on MSS composition (10), some of the early research conducted on cigarette MSS composition, particularly the PAHs, will be described briefly to illustrate the capability and competence of the investigators involved[1]. Complete details of the experimental procedures and findings are available on the Internet at www.rjrtdocs.com.

The initial RJRT PAH investigation involved isolation and identification of 11 PAHs in the MSS from non-filtered cigarettes (11) (Table 1). Naphthalene, anthracene, pyrene, fluoranthene, and B[*a*]P, isolated in crystalline form, were characterized by UV spectral data as well as by classical chemical means (mixture melting point, IR spectra, derivatization, derivative properties). The other six were identified solely on the basis of agreement of their UV spectra with those of authentic samples or with published UV data.

The second investigation involved the MSS from filter-tipped cigarettes (12) (Table 2). Identified were 43 PAHs, including the 11 PAHs identified in the initial study (11). Of the 43 PAHs, 14 were isolated in crystalline form and characterized by both UV spectral and classical chemical means (Table 1). The other 29 were identified from the agreement of their UV spectra with those of authentic

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

Table 1.
Polycyclic aromatic hydrocarbons in cigarette smoke condensate identified by spectral plus other means

Polycyclic aromatic hydrocarbon	PAH				TNF complex		Other Identification
	UV[a]	IR[a]	mp[b]	mmp[c]	mp[d]	mmp[e]	
Naphthalene[f, g]	x	x	x	x	x	x	—
Naphthalene, 1-methyl-[g]	x	—	—	—	x	—	—
Naphthalene, 2-methyl-[g]	x	—	—	—	x	—	—
Acenaphthene[g]	x	—	x	x	x	x[h]	x picrate UV[a] x picrate mp[b]
Acenaphthylene[g]	x	x	x	—	—	—	—
Fluorene[g]	x	—	x	x	x	x	—
Anthracene[f, g]	x	x	x	x	x	x	x picrate IR[a] x picrate mmp[e] x dione IR[a]
Phenanthrene[g]	x	x	x	x	x	x	—
Fluoranthene[f, g]	x	x	x	x	x	x	—
Pyrene[f, g]	x	—	x	x	x	x	—
Chrysene[g, i]	x	—	—	—	x	x	—
Benz[*a*]anthracene[i]	x	—	x	—	x	—	x dione UV[a]
Benzo[*a*]pyrene[f, g, i, j]	x	x	x	x	—	—	x picrate mp[b] x picrate mmp[e]
Dibenz[*a,h*]anthracene[g, i]	x	—	—	—	—	—	x dione UV[a]

[a]Spectrum (UV, IR) of isolate was identical with that of an authentic sample and/or with that reported in the literature.
[b]Melting point (mp) of isolate was identical with that of an authentic sample and/or with that reported in the literature.
[c]Mixture melting point (mmp) of isolate with an authentic sample showed no depression.
[d]Melting point (mp) of TNF (2,4,7-trinitrofluorenone) complex of isolate was identical with that of an authentic sample and/or with that reported in the literature.
[e]Mixture melting point (mmp) of TNF (or picric acid) complex of isolate with TNF (or picric acid) complex of an authentic sample showed no depression.
[f]Spectral plus *other* identification data from RODGMAN (11), a study involving the fractionation of the MSS from 10,500 cigarettes.
[g]Spectral plus *other* identification data from RODGMAN and COOK (12), a study involving the fractionation of the MSS from 20,000 cigarettes.
[h]UV and IR spectra of TNF complex of isolate were identical with those of an authentic sample of acenaphthene:TNF complex.
[i]Reported to be tumorigenic, under certain experimental conditions, to the skin of laboratory animals (13).
[j]Spectral plus melting point data from WYNDER and HOFFMANN (75).

samples or with published spectra. B[*a*]P, benz[*a*]anthracene (B[*a*]A), and dibenz[*a,h*]anthracene (DBA) had been reported to be tumorigenic to mouse skin although the bioassay data for B[*a*]A were contradictory (13,14).

Subsequently, two PAH identifications based solely on UV data were found to be incorrect. One was cholanthrene, a benzocyclopentanthracene (1,2-dihydrobenz[*j*]aceanthrylene). In the massive, definitive study by United States Department of Agriculture (USDA) personnel on the identification of PAHs in MSS, cholanthrene was not among the several benzocyclopentanthracenes reported (15). RODGMAN and COOK (12) obviously assigned an incorrect structure to the PAH they defined as cholanthrene (1,2-dihydrobenz[*j*]aceanthrylene). The other incorrectly characterized PAH was dibenzo[*a,l*]pyrene, initially named 1,2,3,4-dibenzopyrene, now named dibenzo[*def,p*]chrysene. It was reported in MSS not only by RODGMAN and COOK (12) but also by LYONS, LYONS and JOHNSON, WYNDER and WRIGHT, BONNET and NEUKOMM, and PYRIKI (16). For its identification, all relied on a published UV spectrum purportedly that of synthetic dibenzo[*a,l*]pyrene (dibenzo[*def,p*]chrysene). However, in 1966, LAVIT-LAMY and BUU-HOÏ (17) determined that the published spectrum was not that of dibenzo[*a,l*]pyrene but of the isomeric dibenz[*a,e*]aceanthrylene (dibenzo[*a,e*]fluoranthene), generated during the supposed synthesis of dibenzo[*a,l*]pyrene. In 1968, this LAVIT-LAMY and BUU-HOÏ finding was acknowledged by HOFFMANN and WYNDER (18). The authentic dibenzo[*def,p*]chrysene (dibenzo[*a,l*]pyrene) was identified in MSS in 1977 (19), but its MSS level was not reported.

Prior to the actual solution of the question of the presence of PAHs in cigarette MSS, both their presence and a possible means to reduce their MSS delivery level had been discussed in mid-1954 (20). As noted previously (7), the presence of PAHs in MSS should never have been a controversial issue. The suggested means to reduce their MSS level was based on the report by ROFFO (21) that the PAH content and specific tumorigenicity of a destructive distillate of an ethanol-extracted tobacco were reduced from those for the destructive distillate from the control tobacco. ROFFO speculated that the precursors of the tumorigenic PAH components of his distillates were ethanol-soluble phytosterols. Eventually his prediction, as far as it went, was found to be true for cigarette MSS (22,23). Because he was unaware of the presence in tobacco of such long-chained terpenoids as solanesol, iden-

Table 2.
The 97 PAHs reported in tobacco smoke prior to 1964[a]: Those noted by the Advisory Committee (AC) (42), Philip Morris (PM) (43), and R.J. Reynolds Tobacco (RJRT) (11,12,41)

No.	Polycyclic aromatic hydrocarbon	AC	PM	RJRT	No.	Polycyclic aromatic hydrocarbon	AC	PM	RJRT
1	Acenaphthene	D[b]	D	x	48	Dibenzo[*def,mno*]chrysene	D[c]	—	x
2	Acenaphthylene	D	D	x	49	Dibenzo[*def,p*]chrysene[d]	D	D	x
3	Anthracene	D	D	x	50	13*H*-Dibenzo[*a,i*]fluorene	—	D	—
4	Anthracene, alkyl-	—	D	—	51	Dibenzo[*a,c*]naphthacene	—	D	D
5	Anthracene, 9,10-dihydro-	—	—	—	52	Dibenzo[*a,j*]naphthacene	—	D	D
6	Anthracene, methyl-	—	—	—	53	Dibenzo[*de,qr*]naphthacene	—	—	x
7	Anthracene, 2-methyl-	—	D	D	54	Dibenzopyrene	—	—	—
8	Anthracene, 9-methyl-	—	—	x	55	Fluoranthene	D[c]	D	x
9	Azulene	—	D	—	56	Fluoranthene, alkyl-	—	—	x
10	Benz[*j*]aceanthrylene, 1,2-dihydro-[e]	—	—	x	57	Fluoranthene, dimethyl-	—	D	D
11	Benz[*j*]aceanthrylene, 1,2-dihydro-3-methyl-	—	D	—	58	Fluoranthene, 8,9-dimethyl-	—	—	D
					59	Fluoranthene, 8-methyl-	—	D	D
12	Benz[*e*]acephenanthrylene[f]	—	—	D	60	9*H*-Fluorene	D	D	x
13	Benz[*a*]anthracene	D	D	x	61	9*H*-Fluorene, 1-methyl-	—	D	—
14	Benz[*a*]anthracene, 7,12-dimethyl-	—	D	D	62	9*H*-Fluorene, 9-methyl-	—	D	D
15	Benz[*a*]anthracene, 5-methyl-	—	D	x	63	1*H*-Indene	—	—	D
16	1*H*-Benzo[*a*]cyclopent[*h*]-anthracene, 2,3-dihydro-	—	D	x	64	Indeno[1,2,3-*cd*]fluoranthene	—	—	—
					65	11*H*-Indeno[2,1-*a*]phenanthrene[g]	—	—	D
17	9*H*-Benzo[*a*]cyclopent[*l*]-anthracene, 10,11-dihydro-	—	D	x	66	Indeno[1,2,3-*cd*]pyrene	—	—	D
					67	Naphthacene	—	—	D
18	Benzo[*cd*]fluoranthene	—	D	—	68	Naphthalene	—	D	x
19	Benzo[*ghi*]fluoranthene	—	D	x	69	Naphthalene, alkyl-	—	—	—
20	Benzo[*j*]fluoranthene	D	D	x	70	Naphthalene, 1,2-dihydro-4-methyl-	—	—	x
21	Benzo[*k*]fluoranthene	—	D	x	71	Naphthalene, 1,6-dimethyl-	—	—	D
22	5*H*-Benzo[*a*]fluorene	—	—	—	72	Naphthalene, 1,8-dimethyl-	—	D	D
23	11*H*-Benzo[*a*]fluorene	—	D	D	73	Naphthalene, 2,6-dimethyl-	—	—	D
24	11*H*-Benzo[*a*]fluorene, 11-methyl-	—	D	D	74	Naphthalene, 2,7-dimethyl-	—	—	D
25	Benzo[*b*]fluorene	—	D	D	75	Naphthalene, 1-methyl-	—	—	x
26	7*H*-Benzo[*c*]fluorene	—	—	D	76	Naphthalene, 2-methyl-	—	D	x
27	Benzo[*a*]naphthacene	—	D	D	77	Naphthalene, 2-phenyl-	—	—	x
28	Benzo[*rst*]pentaphene	D	—	x	78	Naphthalene, 1,3,6-trimethyl-	—	—	D
29	Benzo[*ghi*]perylene	D[c]	D	x	79	1*H*-Naphtho[3,2,1,8-*defg*]-chrysene	—	—	D
30	Benzo[*c*]phenanthrene	D	D	x	80	Naphtho[2,1,8-*qra*]naphthacene	—	D	X
31	Benzo[*a*]pyrene	D	D	x	81	Pentaphene	—	D	D
32	Benzo[*a*]pyrene, alkyl-	—	D	D	82	Perylene	—	D	x
33	Benzo[*a*]pyrene, 7,8-dihydro-	—	D	D	83	Phenanthrene	D	—	x
34	Benzo[*a*]pyrene, dimethyl-	—	—	—	84	Phenanthrene, dimethyl-	—	—	x
35	Benzo[*a*]pyrene, methyl-	—	D	x	85	Phenanthrene, 2,5-dimethyl-	—	D	D
36	Benzo[*e*]pyrene	D[c]	D	x	86	Phenanthrene, methyl-	—	—	—
37	2,2'-Binaphthylene	—	—	—	87	Phenanthrene, 9-methyl-	—	D	x
39	Chrysene, alkyl-	—	D	x	88	Pyrene	D	D	x
40	Chrysene, dimethyl-	—	D	D	89	Pyrene, alkyl-	—	D	D
41	Chrysene, 1-methyl-	—	D	D	90	Pyrene, ethyl-	—	D	—
42	Coronene	D[c]	D	x	91	Pyrene, methyl-	—	—	—
43	15*H*-Cyclopenta[*a*]phenanthrene	—	—	—	92	Pyrene, 1-methyl-	—	D	x
44	15*H*-Cyclopenta[*a*]phenanthrene, 16,17-dihydro-	—	D	D	93	Pyrene, 2-methyl-	—	D	x
					94	Pyrene, 4-methyl-	—	D	x
45	4*H*-Cyclopenta[*def*]phenanthrene	—	D	D	95	Rubicene	—	—	—
46	Dibenz[*a,h*]anthracene	D	D	x	96	Tribenz[*a,c,h*]anthracene	—	D	D
47	Dibenzo[*b,def*]chrysene	—	D	x	97	Triphenylene	—	—	—
							18	61	77

[a]By January 1964, the date of the report of the Advisory Committee to the Surgeon General, of approximately 2100 PAHs bioassayed in laboratory animals, 480 (22.9%) were found to be tumorigenic to some degree.

[b]D indicates that the specified PAH was discussed in the reference cited; X indicates that the particular PAH was reported in cigarette MSS at RJRT R&D (11,12,41). Note that of the 18 PAHs noted in the Advisory Committee's report, five (fluoranthene, benzo[*ghi*]perylene, benzo[*e*]pyrene, coronene, and dibenzo[*def,mno*]chryene) were not discussed as components of cigarette MSS but were discussed as components of carbon black.

[c]PAHs designated as D[c] in the AC column were those discussed as carbon black components not cigarette MSS components.

[d]The PAH originally identified in cigarette MSS by WYNDER and WRIGHT (76), WYNDER *et al.* (79), LYONS and JOHNSTON (77), LYONS (78), BONNET and NEUKOMM (16), RODGMAN and COOK (12), and PYRIKI (80) as dibenzo[*a,l*]pyrene (dibenzo[*def,p*]chrysene) on the basis of published UV spectral data was subsequently shown to be dibenz[*a,e*]aceanthrylene (17). The authentic dibenzo[*a,l*]pyrene (dibenzo[*def,p*]chrysene) was subsequently identified in MS by SNOOK *et al.* (19) at the USDA.

[e]The PAH originally defined as cholanthrene (1,2-dihydrobenz[*j*]aceanthrylene) by RODGMAN and COOK (12) may have been one of several cyclopentabenzanthracenes later reported by SNOOK *et al.* who identified over 500 PAHs in MSS (15). However, SNOOK *et al.* did not identify cholanthrene in cigarette MSS (19).

[f]The PAH known as benz[*e*]acephenanthrylene was formerly known as benzo[*b*]fluoranthene.

[g]The PAH known as 11*H*-indeno[2,1-*a*]phenanthrene was formerly known as 11*H*-naphtho[2,1-*a*]fluorene.

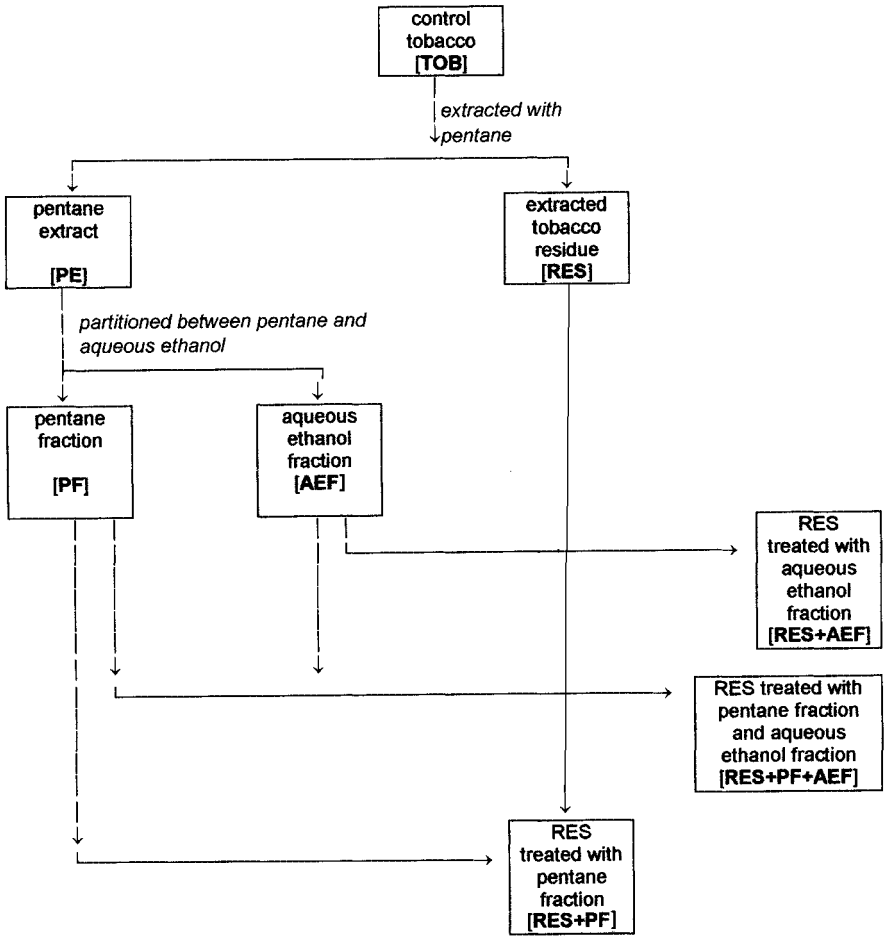

Figure 1.
Tobacco extraction study

fied in flue-cured tobacco in 1957 by ROWLAND *et al.* (24), ROFFO obviously could not include them in his 1942 precursor prediction.

Knowledge of ROFFO's finding on the properties of the destructive distillates from ethanol-extracted *vs* control tobacco was eventually coupled with the findings of LAM (25). LAM reported that the pyrolysis of tobacco long-chained aliphatic hydrocarbons in an inert atmosphere at a temperature approximating that of the burning coal of a cigarette yielded a series of PAHs, ranging from naphthalene to dibenzo[*def,mno*]chrysene (anthanthrene) and including B[*a*]P.

Armed with the knowledge of a) the presence of PAHs in cigarette MSS, b) their possible precursors in tobacco, c) an analytical procedure to quantitate MSS PAHs, and d) the possible effect of solvent extraction on cigarette MSS properties, several aspects of the organic solvent extraction of tobacco were studied. Ultimately incorporated into the process (Figure 1) was an aqueous alcohol-hexane partition step to separate the polar, more flavorful tobacco components from the lipophilic components suspected to be the PAH precursors. In the mid-1950s, little was known about the nature of the polar tobacco components although it was suspected they contributed much to the flavor and aroma of tobacco smoke. The reason for the lack of knowledge about these components was the lack of an adequate fractionation system to separate highly polar compounds in a complex mixture. This situation continued during years of intensive effort on tobacco smoke composition but was finally resolved by SCHUMACHER *et al.* (26) in the 1970s. MSS components identified in their study plus those described by NEWELL *et al.* (27) and HECKMAN and BEST (28) totaled 1545: Of these, 828 were new to tobacco smoke literature, 717 were confirmations of previously reported MSS components. In addition, LLOYD *et al.* (29) identified 323 flue-cured tobacco components (275 new to flue-cured tobacco, 132 new to all tobacco types). Many of these tobacco components were highly polar and potential contributors to MSS flavor and aroma. Subsequently, it became apparent that many of the highly polar components of tobacco and tobacco smoke were identical with or similar to many of the components used in the flavor additive formulations, i.e., the "top dressing", added to a specific tobacco blend to impart its unique smoking characteristics (30).

As an extension of ROFFO's study of the effect of organic solvent extraction of tobacco, the initial organic solvent extraction at RJRT involved pentane extraction of a cased commercial blend cigarette tobacco (TOB) plus

Table 3.
Study of the solvent extraction of tobacco

Sample no.	Designation[a]	Sample composition	Total PAHs	B[*a*]P
I	TOB	Unextracted cased commercial blend tobacco (the control)	$PAH_{Sample\ I}$	$B[a]P_{Sample\ I}$
II	RES	Pentane-extracted cased commercial blend tobacco	$PAH_{Sample\ II} < PAH_{Sample\ I}$	$B[a]P_{Sample\ II} < B[a]P_{Sample\ I}$
III	RES + PF	Pentane-extracted cased commercial blend tobacco (Sample II) to which the appropriate amount of the pentane fraction of the pentane-aqueous ethanol partition was returned	$PAH_{Sample\ III} \approx PAH_{Sample\ I}$	$B[a]P_{Sample\ III} \approx B[a]P_{Sample\ I}$
IV	RES + AEF	Pentane-extracted cased commercial blend tobacco (Sample II) to which the appropriate amount of the aqueous ethanol fraction from the partition was returned	$PAH_{Sample\ IV} \approx PAH_{Sample\ II} < PAH_{Sample\ I}$	$B[a]P_{Sample\ IV} \approx B[a]P_{Sample\ II} < B[a]P_{Sample\ I}$
V	RES + AEF + PE	Pentane-extracted cased commercial blend tobacco (Sample II) to which the appropriate amounts of both the pentane and aqueous ethanol fractions from the partition were returned	$PAH_{Sample\ V} \approx PAH_{Sample\ I}$	$B[a]P_{Sample\ V} \approx B[a]P_{Sample\ I}$

[a]TOB = control tobacco; RES = extracted tobacco residue; PF = pentane fraction; AEF = aqueous ethanol fraction (AEF); PE = pentane, *see* also Figure 1.

solvent partition of the pentane extract (PE) between pentane and aqueous ethanol into a non-polar (pentane-soluble) fraction (PF) and a polar (aqueous ethanol-soluble) fraction (AEF) *(see* Figure 1 and Table 3) (31,32). Five sets of cigarettes were fabricated from the control and pentane-treated tobaccos indicated, machine smoked with the parameters used by WYNDER *et al.* (4), i.e., 35-mL puff volume, 2-sec puff duration, 3 puffs/min in contrast to the usual 35-mL puff volume, 2-sec puff duration, 1 puff/min (33), and the mainstream cigarette smoke condensates (MS CSCs) were analyzed for total PAHs and several individual PAHs, including B[*a*]P. A mixture of 11 PAHs (naphthalene, acenaphthene, anthracene, phenanthrene, pyrene, fluoranthene, chrysene, B[*a*]A, perylene, B[*a*]P, benzo[*ghi*]perylene) was added to CSC in the ratio found in the CSC. The analytical procedure for total PAHs in the CSCs gave about a 98% recovery of the added PAHs. Recoveries of the added 11 individual PAHs in the CSC varied from 92% for acenaphthene to 103% for naphthalene; recovery of the added B[*a*]P was 94% (34).

Analysis of the MS CSCs from the control (TOB) and pentane-extracted tobacco (RES) cigarettes indicated that pentane extraction of the cased commercial tobacco blend resulted in reduction of the level of total PAHs in the MSS on both a per cigarette basis and a per milligram of total particulate matter (TPM) basis. Results of the analyses of the MS CSCs from Samples I–V for PAHs are summarized below and in Table 3.

- Pentane extraction of the cased commercial blend reduced the level of total PAHs in its MS CSC, i.e., $PAH_{Sample\ II} < PAH_{Sample\ I}$.
- Return of the pentane portion (PF) from the pentane-aqueous alcohol partition to the extracted tobacco increased the total PAH level in its MS CSC almost to the level in the control tobacco MS CSC, i.e., $PAH_{Sample\ III} \approx PAH_{Sample\ I}$.
- In contrast, return of the aqueous ethanol portion from the partition to the extracted tobacco had little effect on the total PAH level, i.e., $PAH_{Sample\ IV} \approx PAH_{Sample\ II}$
- Return of both the pentane and aqueous ethanol portions to the extracted tobacco resulted in a MS CSC with a level of total PAH slightly higher, but not significantly so, than that of the control tobacco, i.e. $PAH_{Sample\ V} \approx PAH_{Sample\ I}$.

The B[*a*]P and total PAH data were similar, but several other PAHs gave discordant results. The pattern with hexacyclic benzo[*ghi*]perylene was similar to that of B[*a*]P, but the pattern with the tricyclic PAHs, e.g., anthracene, was not. During this study, the presence in tobacco and tobacco smoke of the duvane (cyclotetradecane) derivatives was not known (35,36). Anthracene and phenanthrene possess the equivalent of a cyclotetradecane structure (Figure 2a). The duvane derivative 12-(1-methylethyl)-1,5-dimethyl-9-methylen-5,8-oxido-3,13-cyclotetradecadien-1-ol is readily convertible to 1,2,5,6,7,8,8a,9,10,10a-decahydro-10a-(1-methylethyl)-5,8-oxido-3,5,8a-trimethylphenanthrene, a decahydrophenanthrene derivative (Figure 2b) (35,36).

In studies of the effect of solvent extraction of tobacco on MSS composition, with particular reference to PAHs, CAMPBELL and LINDSEY (37) also observed the lack of parallelism between changes in the levels of several tetracyclic and higher PAHs in MS CSC from modified tobaccos. Their data plus those of RODGMAN (38) indicated that B[*a*]P is not a valid "marker" or "indicator" for tumorigenic PAHs, tetracyclic or higher, in CSC as claimed by WYNDER and HOFFMANN (39).

The pentane extraction plus the partitioning of the non-polar (pentane) extract between non-polar (pentane) and polar (aqueous ethanol) solvents and the return of the flavorful aqueous ethanol-soluble material to the extracted tobacco was sufficiently unique to result in patent coverage (40).

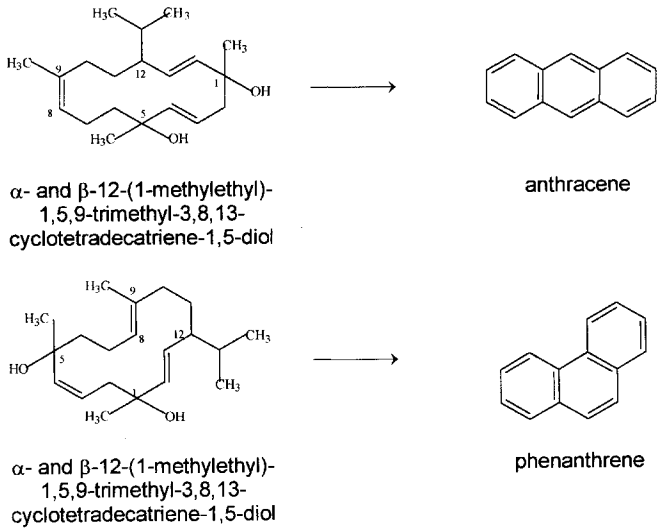

Figure 2a.
Tricyclic aromatic hydrocarbons from cyclotetradecatrienediols

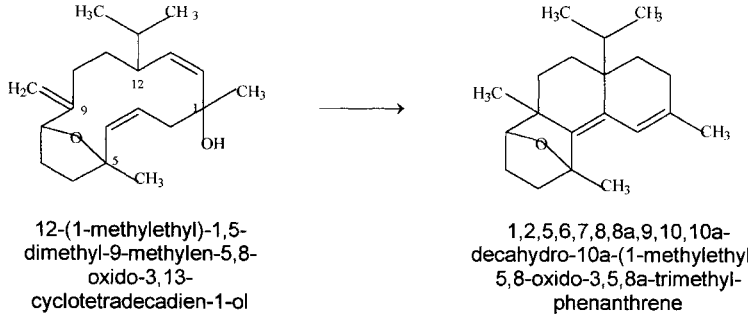

Figure 2b.
A phenanthrene derivative from a cyclotetradecadienol ether

Expert panelists rated the organoleptic properties of Samples I, IV, and V to be similar and much superior to those of Samples II and III. The latter samples were deficient in tobacco components preferentially soluble in aqueous alcohol, i.e., theoretically the flavorful ones. Subsequently, it was confirmed that many were indeed highly flavorful after the development of a procedure that permitted separation and identification of the components of the water-soluble fraction of tobacco and its smoke (26).

During episodes of litigation in the mid- to late 1990s involving state and federal agencies and RJRT and other U.S. Tobacco Industry members, RJRT was repeatedly criticized for its failure to publish its findings on the identification of the 43 PAHs in cigarette MSS, their precursors in tobacco, and the various methods studied whereby their per cigarette delivery levels were reduced. Initially, the goal of the research was to define proprietary methods to accomplish the removal or reduction of MSS components reported to be tumorigenic in laboratory animals, or to be promoters of known tumorigens, or to be ciliastatic in *in vitro* systems. Critics repeatedly cited an in-house February 1964 report by RODGMAN (41) in which over 30 MSS studies conducted between 1955 and late 1963 were summarized.

The 43 PAHs identified in MSS and discussed by RODGMAN (41) were contrasted by critics to the 18 MSS PAHs discussed by the Advisory Committee in its 1964 Report to the U.S. Surgeon General (42) in the tobacco-tobacco smoke chapter, authored by FIESER and ORCHIN. In May 1963, the Advisory Committee management was provided with a highly referenced monograph authored by Philip Morris R&D personnel on reported tobacco and tobacco smoke components (43). Its receipt was acknowledged in an Advisory Committee management-to-Philip Morris management letter but the list of 61 PAHs reported in tobacco smoke was obviously ignored by FIESER and ORCHIN. Ignored by the critics were the differences among the three reports (41–43) in terms of completeness (*see* Table 4). Although the RJRT information on PAHs and phenols was not published for some years because of possible proprietary use, much of it was presented later at several scientific meetings (44) when the possibility of proprietary use no longer existed.

Though they were not discussed during the litigation episodes in the 1990s, differences between the RODGMAN

Table 4.
1964 Discussions of polycyclic aromatic hydrocarbons in cigarette mainstream smoke by RODGMAN (41) and by the Surgeon General's Advisory Committee (42)

PAHs in MSS	No.
Published scientific literature[a]	91
Discussed by RODGMAN (41)[b]	97
Identified by RODGMAN and COOK (12)	43
Identified by RODGMAN and COOK that were discussed in published literature[a]	37
Discussed specifically as tobacco smoke components in Advisory Committee's 1964 report (42)[a]	13
Nontumorigenic PAHs identified in MSS that were noted in a general way but not listed in Advisory Committee's 1964 report (42)[a]	27
Discussed as carbon black components but not tobacco smoke components in Advisory Committee's 1964 report (42)[a]	5
Lliterature citations to late 1963 on identification of MSS PAHs	198
Literature citations to late 1963 on identification of MSS PAHs cited in Advisory Committee's 1964 report (42)	15
Literature citations to late 1963 on identification of MSS PAHs cited in reports by RODGMAN *et al.*	128
Number of additional MSS PAHs reported in published scientific literature between late 1963 and late 1969	14
Number of MSS PAHs discussed by RODGMAN in scientific presentations 1965–1969 (44)	96–110[c]

[a]Because the Advisory Committee's 1964 report issued on January 15, 1964, only literature citations to late 1963 were considered.
[b]Because the RODGMAN report issued in early February 1964, only literature citations to late 1963 were considered.
[c]Number of PAHs discussed depended on the date of the presentations, 1965, 1968, or 1969.

1964 report (41) and the Advisory Committee's 1964 report (42) included:

- Between early 1950s and late 1963 (the cut-off date for the Advisory Committee's 1964 report), at least 97 PAHs had been described as cigarette MSS components. Of these, 91 were reported in the published literature. Six PAHs identified by RODGMAN and COOK (12) had not been reported elsewhere by year-end 1963. However, by 1970, all but one (cholanthrene) of the 97 had been reported in the published literature.
- No mention was made of the 61 PAHs in tobacco smoke listed in the Philip Morris monograph (43).
- No mention was made of the Advisory Committee's comment on 27 nontumorigenic PAHs with four or more fused rings reported as cigarette MSS components. This number plus the 18 noted by the Advisory Committee brought the total number of MSS PAHs acknowledged by the Advisory Committee to 45! From findings reported in the mid-1950s, it was known that several nontumorigenic PAHs in MSS were anticarcinogens when administered with such potent tumorigens as B[*a*]P and DBA.
- No mention was made of five smoke composition presentations at scientific meetings (TCRC) or 14 smoke composition publications in peer-reviewed journals (*Tobacco Science, Journal of Organic Chemistry*) by RJRT personnel.
- No mention was made of the fact that between 1953 and year-end 1963 some 198 articles were published and/or presented at scientific meetings on PAHs in cigarette MSS, their isolation and identification, estimation of their per cigarette MSS delivery level, mechanisms of their formation, their contribution to the observed biological properties in laboratory animals, etc. In his various reports dealing with PAHs, RODGMAN *et al*. referred to 128 of the 198 previously published articles. FIESER (and ORCHIN) in the Advisory Committee Report cited only 15 of them. The omission of so many pertinent references on a specific topic supposedly of great importance in the lung cancer–smoking issue was also characteristic of several subsequent Surgeon General's reports.
- No mention was made that by the mid-1970s over 500 PAHs had been identified in cigarette MSS by USDA personnel (15).

In Table 2 are listed the 97 PAHs with an indication of those discussed in the 1964 Report of the Advisory Committee to the Surgeon General (42) and those listed in the Philip Morris monograph (43). The 43 PAHs identified at RJRT are also indicated in Table 2. Of the 91 PAHs described in the published literature, the Advisory Committee discussed only 18 (19.7%). Of the 97 PAHs known to him, RODGMAN discussed 77 (79.4%) of them in reports issued between 1954 and 1964, including the 43 (44.3%) PAHs identified at RJRT as well as the ones discussed in the 1964 RODGMAN report, a summary of his 34 previous reports on cigarette MSS composition.

III. PROBLEMS ENCOUNTERED IN ATTEMPTS TO CONTROL INDIVIDUAL SMOKE COMPONENTS OR CLASSES OF COMPONENTS

By the early 1960s it was known that attempts to reduce the levels of individual MSS components or classes of components, while successful *per se*, led to unanticipated problems. For example, organic solvent extraction of tobacco removed lipophilic components known or suspected to be precursors of MSS PAHs, the delivery levels of the MSS PAHs were reduced, but the specific tumorigenicity of the CSC from extracted tobacco cigarettes was not reduced proportionately.

Organic solvent extraction of tobacco, while removing lipophilic PAH precursors, increased the levels of lignin, the biopolymeric carbohydrates (cellulose, pectins, starch), and nitrate in the extracted tobacco residue by a factor of 8% to 12%, a factor equivalent to the weight of the lipophilic material extracted. Lignin and the carbohy-

Table 5.
Alteration of cigarette mainstream smoke delivery, composition, and biological activity: methods studied[a]

Cigarette design technology[b]	W&H 1960	W&H 1964	W&H 1965	W&H 1966	W&H 1967	W&H 1969	W&He 1976	NCI 1976–80	HSHW 1978	US SG 1979	W&H 1979	LHHW 1980	US SG 1981	H&W 1986	H&H 1997
Tobacco selection	x	x	x	x	x	x	x	x	x	x	x	—	x	x	x
Type	—	—	x	—	x	x	—	—	—	x	—	x	—	x	x
Stalk position	—	x	—	—	x	x	—	—	—	x	—	—	—	—	
Nitrate content	—	—	—	—	x	x	x	x	x	x	x	x	—	x	x
Nicotine content	—	x	—	—	x		—	—	—	—	—	—	—	—	x
Other components	—	x	—	—	x	x	—	—	—	x	—	—	—	—	x
Tobacco treatment	—	—	x	x	x	x	—	—	—	x	—	—	—	—	x
Curing	—	—	x	x	x	x	—	—	—	x	—	—	—	—	x
Grading	—	—	—	—	x	x	—	—	—	x	—	—	—	—	—
Fermentation	—	—	—	—	x	x	—	—	—	—	—	—	—	—	—
Extraction	—	—	x	—	x	x	x[c]	—	x[c]	x[c]	x[c]	—	—	—	—
Denicotinization	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Ammoniation	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Expansion (lamina and/or stems)[d]	—	—	—	—	—	—	x	x	x	x	x	x	x	x	x
Tobacco additives	—	x	x	x	x	x	x	x	x	x	x	x	—	x	x
Combustion modifiers	—	x	x	x	x	x	x[c]	x[c]	x[c]	x[c]	x[c]	—	—	—	x
Casing materials	—	—	—	—	x	—	—	x	—	—	x	—	x	—	x
Flavorants	—	—	—	—	x	—	—	—	—	—	x	—	x	—	x
Pesticides, agricultural chemicals	—	—	—	—	x	—	—	x	—	—	x	—	x	—	x
Blending[e]	—	—	—	—	x	x	x	x	x	x	x	—	x	—	x
Amount of tobacco	x	—	x	x	x	x	—	—	—	x	—	—	—	—	x
Cigarette dimensions	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Tobacco weight	—	x	—	—	x	—	—	—	—	—	—	—	—	—	—
RTS (nonpaper)	—	—	x	x	x	x	x	x	x	x	x	x	x	x	x
RTS (paper)[f]	—	—	x	x	x	x	x	x	x	x	x	x	x	x	x
Homogenized leaf	—	x	—	—	x	—	—		—	—	—	—	—	—	—
Stem inclusion	—	x	—	x	x	x	x		x	x	x	x	—	x	x
Expanded laminae[d]	—		—	—	—	—	x	x	x	x	x	x	x	x	x
Moisture content	—	x	—	—	x	—	—		—	—	—	—	—	—	—
Tobacco cut width	—	—	x	x	x	x	x	x	x	x	—	x	—	x	x

Table 5 (contd).

Cigarette design technology[b]	W&H 1960	W&H 1964	W&H 1965	W&H 1966	W&H 1967	W&H 1969	W&He 1976	NCI 1976–80	HSHW 1978	US SG 1979	W&H 1979	LHHW 1980	US SG 1981	H&W 1986	H&H 1997
Cigarette paper	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Porosity (air dilution)[g]	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Additives[h]	—	x	—	—	x	—	—	x	—	—	—	—	—	—	x
Coatings	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Filtration	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Efficiency/selectivity[i]	x	x	x	x	x	x	—	x	x	—	—	—	x	x	x
Additives[j]	—	x	—	—	x	x	—	x	x	—	—	—	x	—	x
Material (CA)[i]	—	x	—	—	x	—	—	—		—	—	x	—	—	x
Material (charcoal)	—	—	—	—		—	x	—	x	—	x	x	—	x	x
Air dilution (perforated filter tips)[k]	—	—	—	—	—	—	x	x	x	x	x	—	x	x	x
Diluents (substitutes)	—	—	—	—	—	—	—	x	x	—	x	—	—	—	x
Cytrel®	—	—	—	—	—	—	—	x	—	—	—	—	—	—	x
NSM®	—	—	—	—	—	—	—	x	—	—	—	—	—	—	x
Expanded grains (J10)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Carbon/carbonized filler	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
SSM®	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Other plants (lettuce, peanut hulls, etc.)	—	—	—	—	x	—	—	—	—	—	—	—	—	—	—

[a]W&H 1960 = WYNDER and HOFFMANN (81); W&H 1964 = WYNDER and HOFFMANN (82); W&H 1965 = WYNDER and HOFFMANN (83); W&H 1966 = WYNDER and HOFFMANN (84); W&H 1967 = WYNDER and HOFFMANN (85); W&H 1969 = WYNDER and HOFFMANN (86); W&H 1979 = WYNDER and HOFFMANN (87); W&He 1976 = WYNDER and HECHT (54); NCI 1976–1980 = National Cancer Institute (88); HSHW 1978 = HOFFMANN *et al.* (89); US SG 1979 = USPHS (90); US SG 1981 = USPHS (91); LHHW 1980 = LAVOIE *et al.* (92); H&W 1986 = HOFFMANN and WYNDER (93); H&H 1997 = HOFFMANN and HOFFMANN (94).

[b]RJRT R&D examined the design topic in considerable detail.

[c]Extraction of tobacco classified as "only of academic interest" by WYNDER and HECHT (54) and the Surgeon General (90).

[d]Expanded tobacco laminae incorporated into RJRT products. U.S. patents issued to RJRT personnel in 1970 (95).

[e]The first cigarette containing a blend of flue-cured, burley, and Oriental tobaccos was introduced by RJRT (the 70-mm *Camel*) in 1913. Maryland tobacco was added to blend in 1917. Most cigarettes prior to 1913 were fabricated with 100% flue-cured or 100% Oriental tobacco. The *Camel*-type blend, the so-called American blend, was copied in most countries, exceptions include the U.K. and Canada.

[f]In 1953, *Winston* was the first marketed cigarette with RTS (no added fiber or adhesive) in the blend. By 1958, all U.S. companies were using RTS. RTS had been used previously as cigar wrapper but not in a cigarette blend.

[g]In 1959, RJRT employed increased cigarette paper porosity to lower MSS "tar" and nicotine delivery.

[h]In 1958, RJRT used citrates on cigarette paper for more uniform combustion of tobacco rod.

[i]RJRT introduced the first highly successful filter-tip cigarettes, the *Winston*, in 1953. Cellulose acetate filter tip included triacetin as plasticizer. MSS delivery and composition subsequently controlled by increase in triacetin level.

[j]Triacetin became the prime filter-tip plasticizer. Eventually, the role of triacetin and other plasticizers in the reduction of the levels of phenols and volatile *N*-nitrosamines in MSS was established.

[k]American Tobacco Co. introduced a product with a perforated filter tip.

drates were known precursors of phenols, many of which were classified in the early 1960s as promoters of PAH tumorigenesis. Increasing their level in tobacco increased delivery of MSS phenols. However, subsequent reports that a) almost complete removal of the simple phenols from cigarette MS CSC by selective filtration (plasticized filter tips) produced little change in its specific tumorigenicity to mouse skin (45) and b) phenol, supposedly the most potent promoter of PAH tumorigenicity, inhibited B[*a*]P tumorigenicity to mouse skin (46) offset most of the claims of MSS phenols as promoters. Also, carbohydrates were known precursors of aldehydes (formaldehyde, acetaldehyde, acrolein), ketones, and acids (formic, acetic) reported to be potent *in vitro* ciliastats. Their levels were increased in the MSS from solvent extracted-tobacco cigarettes. Here again, their importance as contributors to respiratory tract cancer induction was substantially diminished when studies by RODGMAN *et al*. (47) and DALHAMN *et al*. (48) in smokers revealed that a large proportion of many of the *in vitro* ciliastats dissolved in the fluids coating the oral cavity and laryngeal area, thus never reaching the ciliated areas of the lung.

While the presence of *N*-nitrosamines (NNAs) in MSS had been predicted (49), their presence in cigarette MSS and the positive relationship between tobacco nitrate level and the NNA levels in tobacco and smoke were not defined till later. Thus, organic solvent extraction of tobacco might be categorized as beneficial because of reduction of mouse-skin tumorigen levels (PAHs) in the MSS but categorized as detrimental because of increase in MSS levels of supposed promoters (phenols), cocarcinogens (phenols), ciliastats (vapor-phase aldehydes, ketones, acids), and organ-specific tumorigens (NNAs).

Unknown at the time was the fact that the extraction also removed tobacco components known to be inhibitors (long-chained aliphatic hydrocarbons) of B[*a*]P tumorigenicity (50) or anticarcinogenic (α-tocopherol, duvanediols) against the potent tumorigens B[*a*]P and DBA (51). Absence or significant depletion of these inhibitors and anticarcinogens from the extracted tobacco was accompanied by substantial reduction of their delivery levels in cigarette MSS. As a result of these factors plus the inordinate expense of extracting nearly a million pounds of tobacco per day, the process of solvent extraction of tobacco followed by partitioning of the extract was abandoned even though several patents (40) were issued on the process.

Other investigators (52) examining the possible utility of the organic solvent extraction of tobacco also abandoned study of the process, classifying it as "impractical both technically and economically" and "practically and economically unfeasible" (53). In 1976, WYNDER and HECHT (54) categorized organic solvent extraction of tobacco as a way to modify the biological effect of its smoke as being "of academic interest only," a sentiment echoed by the U.S. Surgeon General in 1979 (55).

The second method proposed to reduce the level of PAHs in cigarette MSS was the use of "catalysts" to modify the combustion process during the smoking of a cigarette. The most effective "catalysts" were nitrates that during the smoking process generated nitric oxide that interfered with the free radical mechanism defined as one of the mechanisms involved in PAH formation. For several years prior to identification of NNAs in tobacco and tobacco smoke, several ways to increase the blend nitrate level were examined as a means to reduce the tumorigenic PAHs in MSS and the specific tumorigenicity of the MS CSC to mouse skin. Nitrate addition lowered the MSS PAHs, including B[*a*]P (ASHBURN [56]; RODGMAN and COOK [22]; BENTLEY and BURGAN [57]; WYNDER and HOFFMANN [58]; HOFFMANN and WYNDER [18,59]; BRYANT and NORMAN [60]), as well as the MSS phenols (59,61). Because tobacco stems were generally high in nitrate, the addition of stem-based RTS to the blend was also proposed and studied (GORI [62]; BRUNNEMANN *et al*. [63]; ADAMS *et al*. [64]). A third way to increase the nitrate level of the blend was to incorporate high-nitrate tobaccos, a technology examined by MORIE and SLOAN (65); RATHKAMP *et al*. (66), and HOFFMANN *et al*. (67). Because of the subsequent demonstration of the relationship between tobacco nitrate level and the levels of NNAs in MSS (65,68,69), the original proposals were superseded by new ones: Incorporate low-nitrate tobaccos in the blend or remove the nitrates from the tobacco (70).

As mentioned previously, research to reduce the levels of individual MSS components or classes of MSS components was replaced by research to reduce MSS components, both vapor- and particulate-phase components, uniformly across the board as much as possible. Such an approach had been voiced by numerous authorities both within and outside of the Tobacco Industry, e.g., DALHAMN's quote of RYLANDER's 1967 comment (71).

Many approaches were investigated in the attempt to design a "less hazardous" cigarette. Table 5 summarizes not only the numerous technologies studied by Tobacco Industry and non-Industry investigators but also those studied in detail at RJRT, a list eventually reduced to the eight technologies in Table 6. Their chronological impact on sales-weighted cigarette MS "tar" and nicotine is shown in Figure 3. By the early 1960s, several cigarette design technologies developed by the Tobacco Industry and used in commercial products were categorized as significant in their contribution to the "less hazardous' cigarette. Ultimately, the initial four design technologies (tobacco blend, effective and efficient filtration, reconstituted tobacco sheet, air dilution via cigarette paper porosity) were increased to eight. Their significance was recognized in "less hazardous" cigarette design by the National Cancer Institute (NCI)[2] and the U.S. Surgeon General.

[2]All eight cigarette design technologies eventually classified as significant by NCI, U.S. Surgeon Generals, and other investigators on the basis of the 10-year NCI Smoking and Health Program on the "less hazardous" cigarette had been incorporated into one or more U.S. commercial cigarette products prior to the first meeting of the Tobacco Working Group formed in 1968 for the NCI program. In other words, from 1968 to 1978, no new design technology was generated in the NCI Smoking and Health Program on the "less hazardous" cigarette.

Table 6.
Cigarette design technologies recognized as contributing to less hazardous cigarettes[a]

No.	Cigarette design technology[b]	W&H 1960	W&H 1964	W&H 1965	W&H 1966	W&H 1967	W&H 1969	W&He 1976	NCI 1976–80	HSHW 1978	US SG 1979	W&H 1979	LHHW 1980	US SG 1981	H&W 1986	H&H 1997
1	Tobacco blend[c]	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
2	Filter tip[d]	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
3	Filter-tip additive[e]	—	x	x	—	x	x	x	x	x	x	x	x	x	x	x
4	RTS[f]	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
5	Paper additive[g]	—	x	x	—	—	—	—	x	—	x	—	—	—	—	x
6	Air dilution (paper porosity)[h]	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
7	Expanded tobacco[i]	—	—	—	—	—	—	x	x	x	x	x	x	x	x	x
8	Air dilution (filter-tip perforation)[j]	—	—	—	—	—	—	x	x	x	x	x	x	x	x	x

[a]Technologies cited in U.S. Surgeon General's 1979 (90), 1981 (91), and 1982 (96) reports on smoking and health.

[b]Refer to Table 5 for citations for W&H 1960, etc..

[c]First cigarette containing a blend of flue-cured, burley, and Oriental tobaccos introduced by RJRT (the 70-mm *Camel*). Maryland tobacco added to blend in 1917. Most cigarettes prior to 1913 were fabricated from a 100% flue-cured blend or a 100% Oriental tobacco blend. Post-WWI, the *Camel*-type blend, the so-called American blend, was copied in most countries, exceptions included U.K., Canada.

[d]RJRT introduced the first highly successful filter-tip cigarettes, the *Winston*, in 1953.

[e]Cellulose acetate filter tip included triacetin as plasticizer. MSS delivery and composition subsequently controlled by increase in triacetin level.

[f]*Winston* was first marketed cigarette with RTS (no added fiber or adhesive) in the blend. By 1958, all U.S. companies were using RTS. RTS had been used previously as cigar wrapper but not in a cigarette blend.

[g]RJRT was first to use citrates on cigarette paper for more uniform combustion of tobacco rod.

[h]RJRT was first to employ increased cigarette paper porosity to lower MSS "tar" and nicotine delivery.

[i]Expanded tobacco laminae incorporated into RJRT products. U.S. patents issued to RJRT personnel in 1970 (95).

[j]American Tobacco Co. introduced a product with a perforated filter tip.

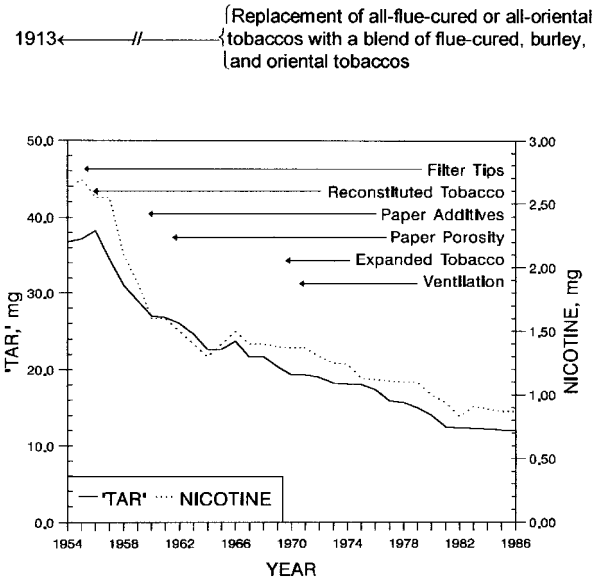

Figure 3.
FTC "tar" and nicotine deliveries, sales weighted average basis

Comments (1960 through 1997) on the significance of these technologies are summarized chronologically in Table 6.

Of course, the initial thrust of this across-the-board reduction was aimed at reducing the MSS "tar" delivery because of extrapolation by WYNDER *et al*. (72) of their 1957 mouse-skin bioassay findings:

> Although it is difficult to estimate a comparable exposure level for man, the human data in line with the animal data indicate that a reduction in total tar exposure will be followed by a decrease in tumor formation. For this reason, measures directed toward this reduction are of utmost importance . . . The minimum dose of tar capable of producing papillomas in mice is about one third, of producing cancer one half, that of the optimum dose . . . The practical implications of these data and their relationship to the human cancer problem have been emphasized.

In his 1957 testimony during the filter-tipped cigarette hearings, WYNDER reiterated this opinion that reducing "tar" exposure dose by 40% to 50% would substantially reduce cancer induction in smokers (73):

Examination of the graphical representation in Figure 3 of the sales-weighted average "tar" delivery for U.S. commercial cigarettes reveals that the 40% to 50% reduction in MSS "tar" delivery considered vital by WYNDER in 1957 was achieved in the late 1960s, i.e., a reduction from 38–39 mg/cig to 19–20 mg/cig. Further examination reveals that by the early 1980s, the sales-weighted average "tar" was further reduced to about 12 mg/cig, i.e., an additional 40% reduction had been achieved. Corresponding reductions in the deliveries of total PAHs in general, B[*a*]P in particular (74), and nicotine were also observed.

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ERRATUM NOTICE

p. 365: Table 2: Ommitted from Table 2 is the following item:

38	Chrysene	−	D	X