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The Composition of Cigarette Smoke: A Chronology of the Studies of Four Polycyclic Aromatic Hydrocarbons*

by

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CONTENTS

SUMMARY

Among the polycyclic aromatic hydrocarbons (PAHs), a major class of identified cigarette mainstream smoke (MSS) components, are several shown to be tumorigenic in laboratory animals and suspect as possible tumorigens to humans. To date, nearly 540 PAHs have been completely or partially identified in tobacco smoke [RODGMAN and PERFETTI (1)]. A detailed chronology is presented of studies on four much discussed PAHs identified in tobacco smoke, namely, benz[*a*]anthracene (B[*a*]A), its 7,12-dimethyl derivative (DMB[*a*]A), dibenz[*a,h*]anthracene (DB[*a,h*]A), and benzo[*a*]pyrene (B[*a*]P). Of the four, DMB[*a*]A, DB[*a,h*]A, and B[*a*]P are considered to be potently tumorigenic on mouse skin painting and subcutaneous injection. Opinions on the tumorigenicity of B[*a*]A to mouse skin vary. DMB[*a*]A is frequently used in tumorigenicity studies as an initiator. Examination of the number of tobacco smoke-related citations listed for these four PAHs reveals the enormous effort devoted since the early 1950s to B[*a*]P vs. the other three. An annotated chronology from 1886 to date describes the tobacco smoke-related research pertinent to these four PAHs, their discovery, isolation and/or identification, quantitation, and contribution to the observed biological activity of MSS or cigarette smoke condensate (CSC). Much of the major literature on these four PAHs in tobacco smoke is presented in order to permit the reader to decide whether the current evidence is sufficient to classify them as a health risk to smokers. There has certainly been a tremendous effort by researchers to learn about these PAHs over the past several decades. Each of these PAHs when tested individually has been shown to possess the following biological properties: 1) Mutagenicity in certain bacterial situations, 2) tumorigenicity in certain animal species, to varying degrees under various administration modes, and 3) a threshold limit below which no tumorigenesis occurs. For more than five decades, it has been known that some of the PAHs, when co-administered in pairs of a potent tumorigen plus a non-tumorigen or weak tumorigen, show inhibitory effects on the tumorigenicity of the most potent, e.g., B[*a*]A plus DB[*a,h*]A; B[*a*]A plus B[*a*]P; anthracene plus DB[*a,h*]A. Over the period studied, some regulatory agencies considered these tobacco smoke PAHs to be serious health concerns, others did not.

With respect to cigarette MSS, certainly the "danger is in the dose" for any MSS component tested singularly to be tumorigenic. But is the level of any of these MSS PAHs high enough to be of concern to smokers? The information herein presented indicates that over the last five decades the following has occurred: 1) The per cigarette yields of these four PAHs have decreased substantially, 2) compared to CSC or Federal Trade Commission (FTC) "tar", their per cigarette yields have also decreased to a point that they may be below any significance biologically, and 3) the specific tumorigenicity in mouse skin-painting studies of the CSC has decreased. These are the three criteria originally proposed to define the "less hazardous" cigarette. Actually, criterion 1) was first directed only at B[*a*]P. Previous studies highlighted the concern that some regulatory bodies

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had in attempting to understand why lung cancer and other forms of cancer seemed more prevalent in smokers. But cigarette smoking alone could not reconcile the evidence. Social, ethnic, environmental, and economic factors are also very important in understanding the entire biological effect. In fact, the level of B[*a*]P in CSC could only explain about 2% of its specific tumorigenicity observed in skin-painted mice and the combination of the levels of all the known tumorigenic PAHs in CSC could only explain about 3% of its tumorigenicity. Despite an 18-month study in the late 1950s, the search for a "supercarcinogen" in MSS and CSC to explain the observed biological effects was unsuccessful. In addition, the exceptional study on MSS PAHs by United States Department of Agriculture (USDA) personnel in the 1970s indicated no "supercarcinogen" was present. Only recently has the concept of complex mixtures in relation to the understanding of the complexity of carcinogenesis taken hold. Perhaps the reason why MSS is less tumorigenic than expected in humans is because of the presence of other MSS components that inhibit or prevent tumorigenesis. For example, it is well known that MSS contains numerous anticarcinogens present in quantities significantly greater than those of the PAHs of concern. When one reviews the history of these four PAHs in MSS or CSC it is clear that many unanswered questions remain. [Beitr. Tabakforsch. Int. 22 (2006] 208–254]

ZUSAMMENFASSUNG

Unter den polyzyklischen aromatischen Kohlenwasserstoffen (PAHs), einer Hauptgruppe der identifizierten Bestandteile des Hauptstromrauchs (HSR) von Zigaretten, gibt es mehrere, deren Tumorgenität in Tierversuchen im Labor nachgewiesen wurde und die als mögliche Tumorgene für den Menschen gelten. Bis heute sind ungefähr 540 PAHs vollständig oder teilweise im Tabakrauch identifiziert [RODGMAN und PERFETTI (1)] worden. In dieser Arbeit wird eine detaillierte Chronologie der Untersuchungen zu den vier der am meisten im Tabakrauch diskutierten PAHs gegeben, nämlich Benz[*a*]anthrazen (B[*a*]A), seinen 7,12-Dimethylderivaten (DMB[*a*]A), Dibenz[*a,h*]anthrazen (DB[*a,h*]A) und Benzo[*a*]pyren (B[*a*]P). Von diesen vier gelten DMB[*a*]A, DB[*a,h*]A und B[*a*]P im Mäusehauttest und bei subkutaner Injektion als stark tumorgen. Die Meinungen bezüglich der Tumorgenität von B[*a*]A auf der Mäusehaut sind unterschiedlich. DMB[*a*]A wird in Tumorgenitätsstudien häufig als Initiator verwendet. Die Zahl der Zitierungen dieser vier PAHs im Zusammenhang mit Tabakrauch seit den frühen 1950er Jahren offenbart die enormen Anstrengungen, die für B[a]P im Vergleich zu den anderen drei PAHs unternommen wurden. Eine kommentierte chronologische Übersicht von 1886 bis heute beschreibt die Forschung über diese vier PAHs im Zigarettenrauch, ihre Entdeckung, Isolierung und/oder Identifizierung, Quantifizierung und ihre Beteiligung an der beobachteten biologischen Aktivität des HSR oder Zigarettenrauchkondensats (CSC). Viele der wichtigsten Arbeiten der letzten vier Jahrzehnte über diese vier PAHs im Zigarettenrauch werden präsentiert, um es dem Leser zu überlassen zu entscheiden, ob der gegenwärtige Kenntnisstand ausreichend ist, um diese PAHs als gesundheitsgefährlich für den Raucher zu klassifizieren. Es wurden ohne Zweifel von Wissenschaftlern in den letzten Jahrzehnten außerordentliche Bemühungen unternommen, mehr über diese PAHs in Erfahrung zu bringen. Die individuelle Testung dieser vier PAHs zeigt, dass sie die folgenden biologischen Eigenschaften aufweisen: 1) Mutagenität in bestimmten Bakterienstämmen, 2) Tumorgenität in unterschiedlichem Maße und nach verschiedenartiger Verabreichung bei einigen Tierspezies, 3) das Vorhandensein eines Schwellenwertes, unterhalb dessen keine Tumorgenität auftritt. Seit mehr als fünf Jahrzehnten ist bekannt, dass bei einigen PAHs bei gleichzeitiger Verabreichung eines starken Tumorgens mit einer nicht tumorgenen oder nur leicht tumorgenen Substanz die tumorgene Wirkung inhibiert wird, z.B. B[*a*]A zusammen mit DB[*a,h*]A; B[*a*]A zusammen mit B[*a*]P; Anthracene zusammen mit DB[*a,h*]A. Im untersuchten Zeitraum sahen einige Überwachungsinstitute diese PAHs im Zigarettenrauch als ernsthaftes Gesundheitsrisiko an, andere taten dies nicht.

Im Hinblick auf den HSR von Zigaretten gilt für jede einzelne als tumorgen getestete Substanz, dass die „Dosis das Gift macht". Ist jedoch die Menge jedes einzelnen im Zigarettenrauch vorkommenden PAHs ausreichend, um ein Risiko für den Raucher darzustellen? Die hier präsentierten Informationen zeigen, dass in den letzten fünf Jahrzehnten Folgendes zu beobachten war: 1) Der Gehalt dieser vier PAHs im Zigarettenrauch hat stark abgenommen, 2) bezogen auf die CSC oder FTC-Menge pro Zigarette haben die PAHs pro Zigarette ebenfalls bis auf eine Menge abgenommen, die unterhalb jeder biologischen Signifikanz liegen könnte, und 3) die spezifische Tumorgenität des CSC beim Mäusehauttest hat abgenommen. Dies sind die drei Kriterien, die ursprünglich vorgeschlagen wurden, um die „weniger schädliche" Zigarette zu definieren. Das Kriterium 1) bezog sich zunächst ausschließlich auf B[*a*]P. Frühere Untersuchungen beschäftigten sich schwerpunktmäßig mit dem Thema, mit dem sich einige Überwachungsbehörden befassten, bei dem Versuch zu verstehen, warum Lungenkrebs und andere Krebsformen bei Rauchern häufiger aufzutreten schienen. Zigarettenrauchen allein schien keine ausreichende Erklärung zu liefern. Soziale, ethische, umweltbedingte und ökonomische Faktoren sind zum Verständnis der gesamten biologischen Wirkung ebenfalls wichtig. So konnte der Gehalt an B[*a*]P im CSC nur ungefähr 2% der spezifischen Tumorgenität im Mäusehauttest erklären und das Zusammenwirken der Konzentrationen aller bekannten tumorgenen PAHs im CSC lieferte für nur 3% seiner Tumorgenität eine Erklärung. Trotz einer 18-monatigen Studie in den späten 1950er Jahren war die Suche nach einem „Superkarzinogen" im HSR und CSC zur Erklärung der beobachteten biologischen Wirkungen nicht erfolgreich. Außerdem zeigte auch die Sonderstudie über das Vorkommen von PAHs im HSR, die von Mitarbeitern des amerikanischen Landwirtschaftsministerium (United States Department of Agriculture, USDA) in den 1970er Jahren durchgeführt wurde, dass kein „Superkarzinogen" existierte. Erst seit kurzem hat sich das Konzept der komplexen Mischungen zum Verstehen der Komplexität der Karzinogenese durchgesetzt. Der Grund, warum der HSR für den Menschen weniger tumorgen ist als erwartet, liegt möglicherweise am Vorhandensein von HSR-Bestandteilen, die die Tumorgenese hemmen oder verhindern. So ist beispielsweise bekannt, dass HSR eine Vielzahl von Antikarzinogenen in Konzentrationen enthält, die signifikant über

denjenigen der betreffenden PAHs liegen. Die Beschäftigung mit der Literatur über diese vier PAHs im HSR oder CSC macht deutlich, dass viele Fragen unbeantwortet bleiben. [Beitr. Tabakforsch. Int. 22 (2006) 208–254]

RESUME

Parmi les hydrocarbures polycycliques aromatiques (PAH), une des classes principales des composants identifiés de la fumée du courant principal (CP), plusieurs se sont montrés tumorigènes chez les animaux de laboratoire et sont suspectés d'être tumorigènes chez l'homme. Jusqu'à présent, 540 PAHs environ ont été identifiés complètement ou partiellement [(RODGMAN et PERFETTI (1)]. Une chronologie détaillée d'études sur quatre PAHs souvent discutés dans la fumée de cigarette, notamment benz[*a*]anthracène (B[a]A), son dérivé de 7,12-diméthyl (DMB[*a*]A), dibenz[*a,h*]anthracène (DB[*a,h*]A) et benz[*a*]pyrène (B[*a*]P). De ces quatre PAHs, DMB[*a*]A, DB[*a,h*]A, et B[*a*]P sont considérés comme fortement tumorigènes sur la peau de souris et après injection sous-cutanée. Des opinions sur la tumorigénicité de B[*a*]A sur la peau de souris diffèrent. DMB[*a*]A est fréquemment utilisé dans des études de tumorigénicité comme initiateur. L'examen du nombre de citations relatives à la fumée du tabac pour ces quatre PAHs révèle l'effort extraordinaire consacré depuis les années 1950 au B[*a*]P par rapport aux trois autres. Une chronologie annotée de 1886 jusqu'à présent décrit la recherche sur ces quatre PAHs présents dans le tabac, leur découverte, isolation et/ou identification, dosage et contribution à l'activité biologique du CP ou du condensat de la fumée de cigarette (CSC). Beaucoup d'études de la littérature sur ces quatre PAHs dans la fumée de tabac sont présentées pour permettre au lecteur de décider si l'évidence est suffisante pour permettre de les classifier comme risque pour la santé du fumeur. Au cours des dernières décennies, des progrès majeurs ont été réalisés dans la recherche sur les PAHs. Chacun de ces PAHs a été examiné individuellement et s'est avéré d'avoir les propriétés biologiques suivantes : 1) mutagénicité dans certaines situations bactériennes, 2) tumorigénicité chez certaines espèces animales, à divers degrés sous divers modes d'administration, 3) une valeur seuil en dessous de laquelle une tumorigénèse ne se manifeste pas. Depuis plus de cinq décennies, il est connu que quelques-uns des PAHs, s'ils sont appliqués sous forme de paires d'un tumorigène fort avec une substance non tumorigène ou légèrement tumorigène, montrent des effets inhibiteurs sur la tumorigénicité des plus forts d'entre eux, comme B[*a*]A plus DB[*a,h*]A; B[*a*]A plus B[*a*]P; anthracène plus DB[*a,h*]A.

Par rapport au CP de la cigarette, pour chaque composant du CP étudié singulièrement par rapport à la tumorigénicité, le « danger est dans la dose ». Mais la teneur de chacun de ces PAHs présent dans le CP est-elle suffisamment élevée pour être dangereuse pour le fumeur ? L'information présentée ci-après indique ce qui a été montré au cours des cinq dernières décennies : 1) Le rendement par cigarette en ces quatre PAHs a fortement diminué, 2) comparé à la teneur en CSC ou en goudron obtenu au moyen de la méthode normalisé de la Federal Trade Commission (FTC), leur rendement par cigarette a également diminué jusqu'à un point où il pourrait être en dessous de toute signification biologique, et 3) la tumorigénicité spécifique du CSC sur la peau de souris a diminué. Ce sont les trois critères proposés pour définir la cigarette « moins dangereuse ». Le critère 1) a au début surtout été appliqué au B[*a*]P. Des études antérieures ont traité le sujet dont se sont préoccupés des autorités réglementaires en essayant de comprendre pourquoi le cancer du poumon et d'autres formes de cancer semblaient être plus fréquentes chez les fumeurs. La fumée de cigarette seule n'a pas permis d'expliquer cette évidence. Des facteurs sociaux, l'origine ethnique, l'environnement et des facteurs économiques sont également très importants pour comprendre l'effet biologique entier. En effet, la teneur en B[*a*]P dans le CSC a seulement expliqué 2% environ de sa tumorigénicité spécifique sur la peau de souris et la combinaison de tous les taux des PAHs tumorigènes connus dans le CSC ont seulement expliqué 3% environ de sa tumorigénicité. Malgré une étude exécutée au cours de 18 mois à la fin des années 1950, la recherche d'un « super carcinogène » dans le CP et le CSC pour expliquer les effets biologiques observés a été sans succès. De plus, l'étude exceptionnelle sur les PAHs dans le CP exécutée par les chercheurs du ministère américain de l'agriculture (United States Department of Agriculture, USDA) dans les années 1970 a révélé qu'aucun « super carcinogène » est présent.

Récemment la conception de mélanges complexes par rapport à la complexité de la carcinogénèse a été adoptée. La raison, pour laquelle le CP est moins tumorigène chez l'homme que celle attendu est peut être dû à la présence d'autres composants du CP qui empêchent ou préviennent la tumorigénèse. Par exemple, il est bien connu que le CP contient de nombreux anti-carcinogènes présents dans des quantités significativement plus élevées que celles des PAHs étudiés. Lors de l'étude de l'histoire des ces PAHs dans le CP ou le CSC, il devient évident que beaucoup de questions se posent encore. [Beitr. Tabakforsch. Int. 22 (2006) 208–254]

1 INTRODUCTION AND BACKGROUND

Since the early 1930s investigators have specifically sought for evidence and identification of polycyclic aromatic hydrocarbons (PAHs) in tobacco smoke [SCHÜRCH and WINTERSTEIN (2), COOPER *et al.* (3), EBY (private communication to KOSAK)] but none was found. Although not benzenoid like its isomer naphthalene, azulene was the first PAH to be isolated and unequivocally identified as a tobacco smoke component by IKEDA in 1947 (4). The identification of azulene has seldom been reported by others despite the enormous effort spent since the early 1950s on identification of components in the PAH fraction from tobacco smoke.

Among the fewer than 100 tobacco smoke components identified by 1954 (5) were several PAHs: azulene, phenanthrene, anthracene, and a "benzopyrene". KOSAK questioned the evidence on which the identifications of phenanthrene, anthracene, and a "benzopyrene" were based. For the "benzopyrene", he cited the 1939 report of ROFFO (6), based on the findings of his son (7). The studies by the ROFFOS did not involve tobacco smoke but a "destructive

distillate" from tobacco, a tobacco-derived entity substantially different from smoke. With respect to the identifications of phenanthrene, anthracene, and "benzopyrene", KOSAK noted in a Footnote:

> These polynuclear hydrocarbons were identified (only some incomplete spectral data were cited) in a material obtained by destructive distillation, rather than the smoking, of tobacco. The work is open to question inasmuch as other investigators who have specifically sought for evidence of the polynuclear aromatics in tobacco smoke have been unable to find any.

Between 1954 and 1960, the number of publications devoted primarily to studies of the PAHs in tobacco smoke increased dramatically (their accumulated number was 10 by mid-1955; 19 by mid-1956; 30 by mid-1958; nearly 70 by late 1959). By late 1963, over 90 PAHs had been identified in cigarette mainstream smoke (MSS) obtained by a smoking procedure *more or less* simulating the human smoking regime [see Table 2 in (8)].

Even today, as in the 1950s and 1960s, most tobacco smoke PAH identifications are based on spectral data (UV, IR, mass, chromatographic retention time) with the most common identification technologies being UV spectral analysis and gas chromatography-mass spectrometry (GC-MS). In an initial study in 1956, RODGMAN (9) identified eleven PAHs in the cigarette smoke condensate (CSC) from a non-filtered cigarette by their UV spectra. In addition, five of the eleven were isolated in crystalline form: Naphthalene, anthracene, pyrene, fluoranthene, and benzo[*a*]pyrene (B[*a*]P) [see Table 1 in (8)]. In a second study conducted between early 1957 and mid-1958, RODGMAN and COOK (10) identified 43 PAHs in MSS from a filter-tipped cigarette. Among the 43 were 14 PAHs (including the preceding five) identified not only by traditional UV spectrophotometry but also by means of classical chemical analysis, e.g., melting point, mixed melting point; derivatization, melting point, and spectral data of derivatives. Benz[*a*]anthracene (B[*a*]A) and dibenz[*a,h*]anthracene (DB[*a,h*]A) were two of the 14 PAHs so identified [see Table 1 in (8)]. Because of their involvement in other PAH-related projects (11, 12, 13, 14), the formal report by RODGMAN and COOK (10) on these PAH results was not completed until mid-1960. In 1959, WYNDER and HOFFMANN (15) reported not only UV spectral data but also the isolation of crystals of B[*a*]P from CSC from 3200 cigarettes. By the late 1970s, SNOOK *et al*. (16, 17, 18, 19) in their massive study of PAHs in tobacco smoke estimated that over 500 tobacco smoke components were PAHs. Less than 2% of the PAHs identified in tobacco smoke have actually been isolated in crystalline form and/or their identity confirmed by the usual physical constants plus derivatization.

Since the early 1950s, several PAHs in tobacco smoke have elicited considerable research effort. These include B[*a*]P, DB[*a,h*]A, and B[*a*]A plus its 7,12-dimethyl-derivative (DMB[*a*]A).

Examination of Section 2 reveals that B[*a*]A has been reported in tobacco smoke over three dozen times. As summarized in Section 3, investigators at only a few laboratories have reported the presence of DMB[*a*]A in tobacco smoke, i.e., PIETZSCH (20, 21); SCASSELLATI-SFORZOLINI and MARIANI (22); and KRÖLLER (23, 24). VAN DUUREN *et al*. (25) examined the initiating effect of DMB[*a*]A and several other tobacco smoke PAHs (benz[*e*]acephenanthrylene, B[*a*]P, chrysene) on the tumorigenicity of mainstream CSC. Several others also investigated the initiating action of DMB[*a*]A and their studies are described in Section 3. Section 4 lists the modest number of reports on DB[*a,h*]A in tobacco smoke. DB[*a,h*]A was the first individual organic compound to generate carcinoma at the site of application in a skin-painted laboratory animal. Section 5 catalogues many, but not all, of the reports on B[*a*]P from over 300 scientists in numerous laboratories. Because of their modest number, Sections 2, 3, and 4 begin with lists of investigators who identified and/or quantitated B[*a*]A, DMB[*a*]A, or DB[*a,h*]A in MSS or otherwise discussed their biological properties, etc. Because of the magnitude of the number of such publications and/or presentations on B[*a*]P, Section 5 does not begin in such a way but similar information is presented chronologically throughout Section 5.

As a class of cigarette MSS components, PAHs in tobacco smoke have long been suspected to be tumorigenic. In a recent review, RODGMAN and PERFETTI (1) listed over 500 identified PAHs in tobacco smoke. Surprisingly, the International Agency for Research on Cancer (IARC) considered the evidence for tumorigenicity in laboratory animals to be sufficient for relatively few, only 27, of the more than 530 PAHs identified in tobacco smoke by 1983 (26) or 1986 (27). Occasionally, IARC reverses its classification of a given PAH, e.g., chrysene, as well its classification of other compounds, e.g., di(2-ethylhexyl) phthalate. Table 1 presents the classification listed by IARC in 1983 and 1986 on the tobacco smoke PAHs identified at that time.

Opinions about the tumorigenicity of B[*a*]A to mouse skin vary considerably: Some authorities classify it as very weakly tumorigenic while others classify it as non-tumorigenic [a]. Others classify its tumorigenicity as "disputed" [DIPPLE *et al.* (28)]. Two of the other three PAHs, DB[*a,h*]A and B[*a*]P, were the first two organic compounds demonstrated to be tumorigenic to mouse skin in 1930 by KENNAWAY and HIEGER (29) and in 1933 by BARRY *et al.* (30), respectively. They and DMB[*a*]A have been rated for many years as three of the four most potent mouse-skin tumorigens used in experimental studies [HARTWELL (31); SHUBIK and HARTWELL (32)]. The fourth is 1,2-dihydro-3-methylbenz[*j*]aceanthrylene (formerly known as 3-methylcholanthrene and sometimes as 20-methylcholanthrene). To date, the identification of this PAH in tobacco smoke has been reported by only one investigator, KRÖLLER (23). Dihydrobenz[*j*]aceanthrylene (cholanthrene) was not among

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One of us (A.R.) while working with the late W.R. Franks at the Banting and Best Department of Medical Research, University of Toronto was involved in 1948–1949 in a comparison of the tumorigenicities of several PAHs (B[*a*]P; DB[*a,h*]A; B[*a*]A) administered by skin painting or by subcutaneous injection. Equimolar doses of each PAH were administered to groups of mice (50 per group) so that the % Tumor Bearing Animals (TBA) with B[*a*]P and DB[*a,h*]A exceeded 80% in both the skin-painted and subcutaneously-injected groups. The equimolar dose of B[*a*]A, a commercial sample, m.p. 166–167 °C, gave only 2% TBA in the skin-painted group, i.e., one mouse with a carcinoma, and 4% TBA in the injected group, i.e., two mice with sarcoma. Purification of the B[*a*]A by sequential complex formation, column chromatography on alumina, and several recrystallizations not only increased the melting point and diminished the m.p. range (167.2–167.5 °C), but improved the UV absorption spectrum. An equimolar dose of the purified B[*a*]A gave 0% TBA; quintupling the dose gave 0% TBA in both the painted and injected groups. The following question remained unanswered: Was the 2% (painted) and 4% (injected) TBA with the commercial sample due to the B[*a*]A or to a contaminant? Unfortunately, the results of the study were never published because of the unwillingness of journals in the late 1940s to accept reports describing negative results.

Table 1. Tobacco smoke polycyclic aromatic hydrocarbons classified by the International Agency for Research on Cancer (26)

Current name	CAS No.	Previous name	Evidence [a]
Benz[*a*]anthracene	56-55-3		sufficient
Benz[*a*]anthracene, 7,12-dimethyl-	57-97-6		[b]
Benz[*a*]anthracene, 5-methyl-	2319-96-2		[b]
Benzo[*j*]fluoranthene	205-82-3		sufficient
Benzo[*k*]fluoranthene	207-08-9		sufficient
Benzo[*rst*]pentaphene	189-55-9	dibenzo[*a,l*]pyrene	sufficient
Benzo[*ghi*]perylene	191-24-2		inadequate
Benzo[*c*]phenanthrene	195-19-7		inadequate
Benzo[*a*]pyrene	50-32-8		sufficient
Benzo[*a*]pyrene, methyl-	25167-89-9		[b]
Benz[*j*]aceanthrylene, 1,2-dihydro-	479-23-2	cholanthrene	[b]
Benz[*j*]aceanthrylene, 1,2-dihydro-3-methyl-	56-49-5	3-methylcholanthrene	[b]
Benzo[*e*]pyrene	192-97-2		inadequate
Benzo[*b*]triphenylene	215-58-7	dibenz[*a,c*]anthracene	limited
Chrysene [c]	218-01-9		limited
Chrysene, 1-methyl-	3351-28-8		inadequate
Chrysene, 2-methyl-	3351-32-4		limited
Chrysene, 3-methyl-	3351-31-3		limited
Chrysene, 4-methyl-	3351-30-2		limited
Chrysene, 5-methyl-	3697-24-3		sufficient
Dibenz[*a,e*]aceanthrylene	5385-75-1	dibenzo[*a,e*]fluoranthene	limited
Dibenz[*a,h*]anthracene	53-70-3		sufficient
Dibenz[*a,j*]anthracene	224-41-9		limited
Dibenzo[*b,def*]chrysene	189-64-0	dibenzo[*a,h*]pyrene	sufficient
Dibenzo[*def,mno*]chrysene	191-26-4	anthanthrene	limited
Dibenzo[*def,p*]chrysene	191-30-0	dibenzo[*a,l*]pyrene	sufficient
Fluoranthene, 2-methyl-	33543-31-6		limited
Indeno[1,2,3-*cd*]pyrene	193-39-5		sufficient
Naphtho[1,2,3,4-*def*]chrysene	192-65-4	dibenzo[*a,e*]pyrene	sufficient
Naphtho[2,1,8-*qra*]naphthacene	196-42-9		inadequate
Phenanthrene, 1-methyl-	832-69-9		inadequate
Triphenylene	217-59-4		inadequate

[a] IARC rating (26,27) of evidence indicating carcinogenicity to laboratory animals.
[b] Not reviewed by IARC (26); not listed in the Cumulative Index in (26) to previous IARC monographs on the evaluation of carcinogens.
[c] The classification of chrysene was recently changed from limited to inadequate.

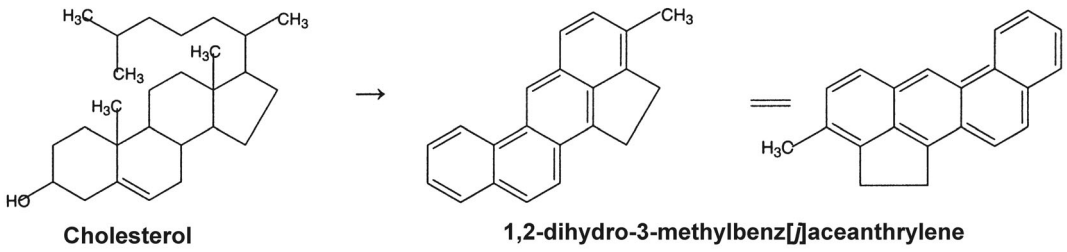


Figure 1. Theoretical conversion of cholesterol to 1,2-dihydro-3-methylbenz[*j*]aceanthrylene

the several PAHs isomeric with 1,2-dihydrobenz[*j*]aceanthrylene reported by SNOOK *et al.* (17, 18, 19). In the late 1940s there was much interest in 1,2-dihydro-3-methylbenz[*j*]aceanthrylene because of its possible generation from cholesterol during the heating of cholesterol-containing foodstuffs (Figure 1).

While 1,2-dihydro-3-methylbenz[*j*]aceanthrylene could actually be synthesized from cholesterol by a series of sophisticated chemical reactions (33), attempts to generate it by pyrolysis of cholesterol were unsuccessful.

Cholesterol and several similarly structured phytosterols (campesterol, β-sitosterol, stigmasterol) are components of tobacco and a portion of each is transferred intact to smoke during the smoking process. All are also present in tobacco and tobacco smoke as glycosides (34) and long-chained aliphatic acid esters (35). Theoretically, all could yield 1,2-dihydrobenz[*j*]aceanthrylene and/or 1,2-dihydro-3-methylbenz[*j*]aceanthrylene during the smoking process.

Some readers may be unfamiliar with the history of PAHs and the numerous studies conducted on their purported

Table 2. Lung cancer death rates and content of air of benzo[*a*]pyrene in communities near Liverpool

Community	Type	B[*a*]P content, μg/m^3 air	Lung cancer death rates [a]
Conway Valley	Village	0.1	59
Llangefni	Village	0.3	53
Ruthin	Small town	0.5	15
Blaenau	Town	0.7	62
Flint	Industrial town	1.85	74
Ormskirk	Industrial town	2.2	95
Hoylake	Resort city	0.3	98
Wrexham	Industrial town	1.95	78
Chester	Industrial town	1.45	112
Bootle	Liverpool suburb	3.75	146
Warrington	Industrial town	4.4	115
St. Helens	Industrial town	4.75	111
Birkenhead	Harbor town	3.3	132
Liverpool	Harbor and industrial town	2.95–6.75	158

[a] Standardized lung cancer death rate 1950–1954; expected rate = 100.

pertinence to cancer induction in humans. The following prelude to Sections 2 through 5 is a brief account of the history of PAHs up to the time of their identification in tobacco smoke which, coupled with epidemiological data on the association of cigarette smoking and lung cancer, led to numerous discussions and assertions of their possible involvement in cancer induction in smokers.

For over two centuries attempts to induce cancer in laboratory animals by administration via skin painting or feeding of various materials had failed until the mid 1910s when YAMAGIWA and ICHIKAWA (36) successfully induced carcinoma on the ears of rabbits skin-painted with a coal-tar solution by prolonging the periodic painting to many months beyond the time limit used in the unsuccessful earlier studies. Their result and procedure led to many studies over the next decade and a half on the cancer-producing properties of various tars, oils, shale oils, soots, products from the pyrolysis of acetylene or isoprene (37), extractable material from various heated foodstuffs [coffee (38), tea (39), yeast (40), meat (41)] or food components, e.g., cholesterol (42). In nearly every instance, carcinomas were produced on the skin-painted animals. During this period, the term carcinogenesis was generated in 1923 and defined simply as "the production of carcinoma". This definition, listed in the 13th Edition of Dorland's Medical Dictionary in 1927, remained unchanged in the 27th Edition of Dorland's Illustrated Medical Dictionary in 1985. As noted previously (43), many investigators incorrectly apply the terms carcinogenesis, carcinogen, and carcinogenicity to the induction of any type of tumor not just to carcinoma. The next step after the induction of cancer in laboratory animals with various tars and/or pyrolysates was extensive research to determine the causative agent(s) in the cancer-causing material.

With DB[*a*,*h*]A, a PAH synthesized in the late 1920s, KENNAWAY and HIEGER (29) induced cancer in mice skin-painted with a solution of it. A few years later, COOK *et al.* (44) isolated and characterized B[*a*]P from coal tar and demonstrated its carcinogenicity in skin-painted mice (30). These two results, the first ever with individual compounds, led to the analysis of many substances such as tar, oil, shale oil, soot, pitch, carbon black, and the pyrolysis product from several materials to determine the causative agent in them. In almost every instance, B[*a*]P, B[*a*]A, and DB[*a*,*h*]A were found. Because of its discovery in coal tar and its tumorigenic potency, B[*a*]P became the prime target of many searches in carcinogenic materials arising from the heating of any organic material. B[*a*]P was determined in shale oil in 1943 (45), in air pollutants in 1953 (46), in cracked petroleum in 1955 (47), and eventually in tobacco smoke (9, 15). The next steps involved detailed studies of air pollutants and their relationship to cancer in the human population. Literally dozens of publications, presentations, and books by such authorities as KENNAWAY [b] in the U.K. and HUEPER (48) in the U.S. defined the toxicants in air pollution and their association with respiratory tract problems, including cancer. An example of the type of data collected on B[*a*]P as an air pollutant component and its relationship to lung cancer incidence is shown in Table 2 from STOCKS (49). Unfortunately, with the advent of the studies of PAHs in tobacco smoke and the concentration on the per cigarette yield of B[*a*]P and other PAHs, the importance of much of the extraordinary research on the adverse effect of the components of air pollution on health has been dismissed.

An interesting comparison is possible when one examines the various reports on the exposure to B[*a*]P from diet. MAGA (50), citing the findings of VAESSEN *et al.* (51), and WALDMAN *et al.* (52) estimated the daily exposure to B[*a*]P via diet to be 500 ng. HATTEMER-FREY and TRAVIS (53) reported the daily exposure to B[*a*]P to be even higher, 2200 ng, and estimated that 97% of such daily exposure to B[*a*]P was by food intake, the remaining 3% by inhalation. Using a different approach, KAZEROUNI *et al.* (54) determined the level of B[*a*]P in 200 food items and estimated the intake of B[*a*]P from consumption of a medium portion size of the various food items.

By use of the per cigarette MSS yield of B[*a*]P from the 1R4F Reference cigarette, the "cigarette equivalent" of the food exposure may be calculated. RODGMAN and GREEN (55) described the B[*a*]P yield data obtained on the 1R4F Reference cigarette in three different laboratories. The average, 5.2 ng/cig, of these data was tabulated by RODGMAN (43). Shown in Table 3 are some meaningful "cigarette equivalents" of total daily exposures or exposure to a medium size portion of several food items.

In the mid-1950s, several histories up to that time were prepared on the early studies on chemical carcinogenesis (56), carcinogens in foodstuffs (57), air pollution carcinogens (58), and carcinogens in various industrial tars, oils, and smokes (59). Throughout the manuscripts, the involvement of PAHs was emphasized because of their ubiquity. In 1956, the discovery of the tumorigenicity of *N*-nitrosamines (NNAs) was reported by MAGEE and BARNES (60). Eventually, as with the PAHs, studies were begun on the presence of volatile and nonvolatile NNAs in various foods, cosmetics, rubber products, and pharmaceuticals.

In 2001, RODGMAN discussed the 97 PAHs that had been reported in tobacco smoke by late 1963 [see Table 2 and accompanying text in (8)]. Of the 97 PAHs, 91 had been

[b] In 1947, E.L. KENNAWAY was knighted for his pioneer research on carcinogenesis and his contributions to the knowledge of the relationship between exposure to environmental tumorigens and cancer.

Table 3. "Cigarette equivalent" of daily exposure to benzo[*a*]pyrene or exposure to benzo[*a*]pyrene in a medium size portion of specific food items

Investigator (reference)	Medium size portion of	B[*a*]P content	Exposure to B[*a*]P, ng [a]	"Cigarette Equivalent" [b]
Maga (50); Vaessen *et al.* (51)	—	—	500	96 (4.8) [c]
Waldman *et al*. (52)	—	—	500	96 (4.8)
Hattemer-Frey and Travis (53)	—	—	2200	423 (21.2)
Kazerouni *et al*. (54)	Grilled steak	532	—	102 (5.1)
"	Barbecued hamburger	129	—	25 (1.25)
"	Barbecued chicken	439	—	84 (4.2)
"	Cream of wheat	50	—	10 (0.5)
"	Oatmeal	29	—	5
"	Grits	27	—	5
"	Spaghetti	22	—	4
"	French fries	22	—	4
"	Pumpkin pie	63	—	12 (0.6)
"	Popcorn	16	—	3
"	Yogurt, frozen	41	—	8
"	Rice	16	—	3
"	Tomato	23	—	4
"	Potato	16	—	3
"	Orange	23	—	4
"	Banana	19	—	4

[a] Estimated total daily exposure to B[*a*]P, ng.
[b] 1R4F "Cigarette Equivalent" at 5.2 ng/cig of B[*a*]P.
[c] Number in parentheses represents number of packs at 20 cig/day, per cigarette MSS yield of B[*a*]P = 5.2 ng.

reported in the published literature. Table 4 lists PAHs identified in both cigarette MSS and automobile exhaust gas at that time. As indicated, several had been shown to be tumorigenic to mouse skin. Three of the PAHs, the subject of this report, are listed in italics. During the period from the mid-1950s to late 1963 when the study of PAHs in tobacco smoke was extremely intense, the MSS PAHs, both mouse-skin tumorigens and nontumorigens, were discussed extensively in the literature as possible causes of adverse health problems in smokers whereas little was written about the same PAHs in automobile exhaust gas.

It should also be remembered that in the early 1950s, research on the tumorigenicity of PAHs had been underway for only a little over two decades with, however, a six-year sojourn from 1939 through 1945 due to World War II (WWII). Most of the tumorigenesis investigators in the U.K., U.S., Canada, France, Russia, Germany, Japan, and Italy were otherwise involved in significant war-related research activities [c].

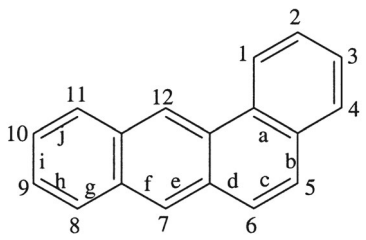
Another important aspect of the early research on PAH tumorigenicity was the following: Because of the lack of knowledge of the existence or nature of the metabolites of such PAHs as B[*a*]P and DB[*a,h*]A and their importance in cancer induction, many conclusions made in the mid-1950s on the tumorigenicity of PAHs were subsequently modified as the metabolite data base expanded.

This report contains a detailed chronology of studies on four PAHs (B[*a*]A, its 7,12-dimethyl derivative DMB[*a*]A, DB[*a,h*]A, and B[*a*]P) identified in tobacco smoke. Of the four, the latter three are considered to be potently tumorigenic to mouse epidermis. Examination of the number of citations listed for these four tobacco smoke PAHs reveals the enormous effort devoted to B[*a*]P vs. the other three PAHs, two of which are considered to be in the same tumorigenicity category as B[*a*]P.

This is an annotated chronology of reports from the late 1880s to date that describe the tobacco smoke-related research pertinent to the four PAHs B[*a*]A, DMB[*a*]A, DB[*a,h*]A, and B[*a*]P, their discovery, isolation and/or identification, quantitation, and contribution to the observed biological activity of MSS or CSC as well as their biological responses to various antitumorigenic compounds (many of which are present in tobacco smoke).

2 ANNOTATED CHRONOLOGY OF BENZ[*a*]ANTHRACENE (B[*a*]A)

The International Union of Pure and Applied Chemistry (IUPAC) numbering and lettering of B[*a*]A and anthracene are as follows:



Benz[*a*]anthracene [$C_{18}H_{12}$] [228], CAS No. 56-55-3

[c] When WWII broke out in 1939, Sir F.G. Banting, University of Toronto, 1923 Nobel Laureate for his discovery of insulin, postponed his cancer research to become liaison officer between the medical branches of the British and Canadian armed forces. In February 1941, he was killed in an airplane crash in Newfoundland. Suspending his cancer research in 1939, W.R. Franks, University of Toronto, spent WWII in the RCAF medical branch. He invented the famous Franks Flying Suit to prevent pilot blackout, designed the human centrifuge to study the effect of increased gravity on humans, and made other contributions to aviation medicine. During WWII, L.F. Fieser, Harvard University, synthesized and screened over 300 compounds, primarily quinones, as antimalarial agents. Although some appeared promising, none proved to be clinically effective.

Table 4. Polycyclic aromatic hydrocarbons identified in cigarette mainstream smoke (MSS) and automobile exhaust (AE) by late 1963

Polycyclic aromatic hydrocarbon	CAS Reg.	MSS	AE
Anthracene	120-12-7	x	x
Benz[*e*]acephenanthrylene[a]	205-99-2	x	x
Benz[a]anthracene[c]	56-55-3	x	x
Benz[*a*]anthracene, alkyl-[a]	—	x	x
Benzo[*ghi*]fluoranthene	203-12-3	x	x
Benzo[*j*]fluoranthene[a]	205-82-3	x	x
Benzo[*k*]fluoranthene[a]	207-08-9	x	x
11*H*-Benzo[*b*]fluorene	243-17-4	x	x
Benzo[*ghi*]perylene	191-24-2	x	x
Benzo[a]pyrene[a]	50-32-8	x	x
Benzo[*a*]pyrene, alkyl-	—	x	x
Benzo[*e*]pyrene	192-97-2	x	x
Chrysene [a, b]	218-01-9	x	x
Chrysene, alkyl-	—	x	x
Coronene	191-07-1	x	x
Dibenz[a,h]anthracene	53-70-3	x	x
Dibenzo[*def,mno*]chrysene	191-26-4	x	x
Dibenzo[*def,p*]chrysene[a]	191-30-0	x	x
Dibenzo[*a,j*]naphthacene	227-04-3	x	x
Dibenzo[*b,pqr*]perylene	190-95-4	—	x
Fluoranthene	206-44-0	x	x
Fluoranthene, alkyl-	—	x	x
Naphthacene	92-24-0	x	x
Naphtho[1,2,3,4-*def*]chrysene	192-65-4	x	x
Pentaphene	222-93-5	x	x
Perylene	198-55-0	x	x
Phenanthrene	85-01-8	x	x
Pyrene	129-00-0	x	x
Pyrene, alkyl-	—	x	x
Triphenylene	217-59-4	x	x

[a] Reported to be tumorigenic to mouse epidermis in late 1963.
[b] Some years after 1963, removed from IARC carcinogen classification.

Investigators who have studied and/or discussed the presence of B[*a*]A in tobacco smoke include: LETTRÉ and JAHN (61), LYONS (62), RODGMAN (8, 9, 11, 63, 64, 65), BONNET and NEUKOMM (66, 67), GILBERT and LINDSEY (68, 69), LYONS and JOHNSTON (70), NEUKOMM and BONNET (71), AHLMANN (72), PIETZSCH (20, 21), WYNDER *et al.* (73), LINDSEY (74, 75), PYRIKI (76), WYNDER and HOFFMANN (15, 77), HOFFMANN and WYNDER (78, 79), PYRIKI *et al.* (80), RODGMAN and COOK (10, 81, 82), WYNDER (83), SCASSELLATI-SFORZOLINI and MARIANI (22), PYRIKI (84), KRÖLLER (24), AYRES and THORNTON (85), PYRIKI (86), KRÖLLER (24), AYRES and THORNTON (87), GUVERNATOR *et al.* (88), KIRYU and KURATSUNE (89), CARUGNO and ROSSI (90), STAMEY and DOBBINS (91), PAPPAS and BINOPOULOS (92), GORI (93, 94), ELMENHORST and GRIMMER (95), KLIMISCH and KIRCHHEIM (96), SEVERSON *et al.* (97), SNOOK *et al.* (17, 18, 19), TSO and CHAPLIN (98), KLUS and KUHN (99), RISNER (100, 101, 102), and GUERIN *et al.* (103).

The following is a list of some of the catalogues of tobacco and/or tobacco smoke components that included B[*a*]A: ROBERTS *et al.* (104), BENTLEY and BERRY (105), JOHNSTONE and PLIMMER (106), PHILIP MORRIS (107), ELMENHORST and RECKZEH (108), STEDMAN (109), and ISHIGURO and SUGAWARA (110).

The following includes articles in which various smoke components, including B[*a*]A, are listed and/or discussed as significant toxicants: USPHS (111, 112, 113), IARC (27), HOFFMANN and WYNDER (114), HOFFMANN and HECHT (115), HOFFMANN *et al.* (116, 117), HOFFMANN and HOFFMANN (118, 119, 120), FOWLES and BATES (121), RODGMAN and GREEN (55), and RODGMAN (43):

1886 ELBS (122) synthesized B[*a*]A from naphthalene and phthalic anhydride.

1927 MAYNEORD [cf. KENNAWAY(123)] reported that solutions of many carcinogenic mixtures, tars, and oils exhibited the same characteristic UV fluorescence spectrum, a spectrum similar to those of PAHs. In mouse skin-painting studies, it was found that the carcinogenicity of the various oils and tars was proportional to the UV fluorescence.

1929 FIESER and DIETZ (124) synthesized B[*a*]A from *o*-toluylnaphthalene.

1930 In the search for a PAH with a UV fluorescence spectrum similar to those observed by MAYNEORD, HIEGER (125) discovered that the UV fluorescence spectrum of B[*a*]A was similar to those of the various carcinogenic tars and oils but differed in that B[*a*]A fluoresced at a slightly longer wavelength.

1932/1933 By following the increase in UV fluorescence as the active ingredient(s) in coal tar were concentrated during fractionation, COOK *et al.* (44) isolated several tetracyclic and pentacyclic PAHs. Among them was a new, potent mouse-skin carcinogen which COOK *et al.* demonstrated, by synthesis, to be B[*a*]P. B[*a*]A and B[*e*]P were two other PAHs isolated from coal tar and identified by COOK *et al.*

1933 HAWORTH and MAVIN (126) reported an alternate synthesis of B[*a*]A from phenanthrene and succinic anhydride.

1951 Most of the tumorigenicity studies summarized by HARTWELL (31) indicated that B[*a*]A was either nontumorigenic or at best a very weak tumorigen. In the 16 studies conducted from 1930 through 1945, involving over 400 laboratory animals, either skin painted or injected, only two epitheliomas were reported, one in each of two separate studies involving a total of 110 laboratory animals [cf. the 1964 ADVISORY COMMITTEE's report to the U.S. Surgeon General [USPHS (127)]. STEINER and FALK (128) demonstrated that, in subcutaneous injection studies, B[*a*]A reduced the tumorigenic effect of the potent tumorigens DB[*a,h*]A and 1,2-dihydro-3-methylbenz[*j*]aceanthrylene.

1953/1955 In his review of the relationship between electronic configuration and tumorigenicity, COULSON (129) listed the carcinogenicity of B[*a*]A to mouse skin as + and its sarcogenicity on subcutaneous injection as + despite his comment that the calculations of the total K region electronic charge according to the PULLMAN and PULLMAN theory (130) for B[*a*]A and many of its methyl and multimethyl derivatives were "far from being satifactory".

1955/1956 FALK and KOTIN (131) reported the per cigarette yields of B[*a*]A and B[*a*]P in MSS, sidestream smoke (SSS), and the cigarette butt.

1955/1958 Various investigators described the identification and level of B[*a*]A in tobacco smoke. In the first few years of the research on the nature of the PAHs in CSC, several significant papers were published, these included: LETTRÉ and JAHN (61), LYONS (62), BONNET and NEUKOMM (66, 67), RODGMAN (9, 11, 63), GILBERT and LINDSEY (68, 69), LYONS and JOHNSTON (70), AHLMANN (72), PIETZSCH (20), and WYNDER *et al.* (73)

1956 In the lengthy review of their studies on the relationship between the tumorigenicity of the angular benz[*a*]acridine and benz[*c*]acridine, LACASSAGNE *et al*. (132) used the properties of the PAH B[*a*]A for comparison.

1957 SHUBIK and HARTWELL (32) listed nine bioassays conducted on the tumorigenicity of B[*a*]A between 1948 and 1952. In one study involving 360 laboratory animals subcutaneously injected with B[*a*]A, 85 developed sarcomas.

1959 JOHNSTONE and PLIMMER (106) in their review on the composition of tobacco and tobacco smoke listed B[*a*]A as a PAH in tobacco smoke.

1960 RODGMAN and COOK (10) reported the isolation of crystalline B[*a*]A from the MSS of a filter-tipped cigarette.

HOFFMANN and WYNDER (79) reported the yield of B[*a*]A, B[*a*]P, and DB[*a,h*]A from the CSC obtained by smoking 430 commercial American non-filtered cigarettes.

1963 HOFFMANN and WYNDER (133) reported that, in a mouse skin-painting study, co-administered B[*a*]A reduced the tumorigenic activity of B[*a*]P.

WYNDER and HOFFMANN (134) described their fractionation scheme for the concentration and determination of the levels of the PAHs in tobacco smoke. They listed B[*a*]A as a carcinogenic PAH and its cigarette MSS yield as 3 ng/cig. This delivery should be contrasted with the delivery range listed by HOFFMANN and WYNDER (114) of 40–70 ng/cig and HOFFMANN and HECHT (115) of 20–70 ng/cig in their lists of "tumorigens in tobacco and tobacco smoke". The PAH tabulation presented in this 1963 publication (134) was that used in the WYNDER-HOFFMANN 1964 review [see p. 319 in (77)] and the WYNDER-HOFFMANN 1967 book [see p. 338 in (135)] on tobacco and tobacco smoke.

1964 In its tabulation of the carcinogenic polycyclic compounds isolated from cigarette smoke, the ADVISORY COMMITTEE in its report to the U. S. Surgeon General did not list B[*a*]A [see Table 2, p. 58 in (127)]. The COMMITTEE discussed B[*a*]A as a tobacco smoke component as follows:

> Benz[*a*]anthracene, identified in cigarette smoke, is very weak or inactive in initiating malignant growth by itself, but initiates carcinogenesis under the influence of croton oil as promoter . . .

WYNDER and HOFFMANN (77) reported that mouse-skin painting with a solution of B[*a*]A plus painting with "tobacco tar" or painting with a solution of "tobacco tar" to which B[*a*]A had been added to the "tobacco tar" did not alter significantly the specific tumorigenicity of the "tar".

HANSCH and FUJITA (136) in their study of the correlation of biological activity and chemical structure listed the Log *P* of B[*a*]A. However, they did not define the tumorigenicity of B[*a*]A. *P*, the partition coefficient of a compound between 1-octanol and water, is considered as an approximation of the partitioning between cytosol and lipid membranes of living systems.

1965 BADGER *et al.* (137) identified numerous PAHs, including B[*a*]A, in the pyrolysate of various tobacco components.

1968 STEDMAN (109) in his review of the composition of tobacco and tobacco smoke listed B[*a*]A as a PAH component of tobacco smoke.

HOFFMANN and WYNDER (138) listed B[*a*]A in MSS at 3 ng/cig, as a tumor initiator, and as a carcinogen with mouse-skin carcinogenicity equivalent to that of indeno[1,2,3-*cd*]pyrene and benzo[*c*]phenanthrene.

1968/1980 The NCI study [GORI (93, 94), NCI (139)] on the design of "a less hazardous cigarette" provided much data to indicate that B[*a*]P was not a valid "indicator" in cigarette MSS for tumorigenic PAHs or PAHs with four or more fused rings. The invalidity of this "indicator" concept was demonstrated by the great variation in the B[*a*]P:B[*a*]A ratio for the MSSs from more than 100 control and 30 test and reference cigarettes. In the NCI study of the first and second set of cigarettes (93), the per cigarette MSS yields of only three PAHs were quantitated: B[*a*]A, B[*a*]P, and phenanthrene. Analyses for phenanthrene in MSS were discontinued in the NCI study of the third and fourth set of cigarettes (94).

1969/1970 GREEN *et al.* (140) identified several PAHs, including B[*a*]A, in the MSS from cigarettes made from several tobacco substitutes, e.g., Cytrel®, Sutton Smoking Material, and puffed grain.

1974 HOFFMANN *et al.* (141) reported that a non-filtered U.S blend cigarette, "at a puff regime of 2 puff/min", delivered 81 ng/cig of B[*a*]A and 47 ng/cig of B[*a*]P in the MSS, per cigarette yields less than those from a bidi cigarette smoked under identical conditions.

1979 The 1979 Surgeon General's report (111) listed B[*a*]A as a tumorigenic PAH in cigarette MSS.

RINKUS *et al.* (142) reported that several cigarette MSS components, including B[*a*]A, were mutagenic in the Ames test (*Salmonella typhimurium*).

1980 BARTSCH *et al.* (143) also reported that several cigarette MSS components, including B[*a*]A, were mutagenic in the Ames test (*Salmonella typhimurium*). They also reported on the mutagenicity of several diol-epoxide metabolites of B[*a*]A.

1984 DIPPLE *et al.* (28) discussed the activity of several of the diol, epoxides, and diol-epoxide metabolites of B[*a*]A (see Section V, 1984 on B[*a*]P for additional remarks on such PAH metabolites).

1985/1986 As a result of its 1985 meetings, the IARC (27) listed B[*a*]A as a biologically active agent present in non-filtered cigarette MSS and listed its yield as 40 to 70 ng/cig. In its tabulation on the evaluation of the carcinogenicity of various tobacco smoke components, IARC listed the degree of evidence for carcinogenicity of B[*a*]A in animals as "Sufficient evidence". No specific comment was included on the degree of evidence regarding the carcinogenicity of this PAH in humans.

1990 HOFFMANN and HECHT (115) used the 1986 IARC data (27) to generate a list of 43 significant "tumorigens" in tobacco and tobacco smoke. Among the eleven PAHs included was B[*a*]A. The HOFFMANN-HECHT list was cited by the Surgeon General in his 1989 report (144) and subsequently used by the Environmental Protection Agency (EPA) (145) in its attempt to have environmental tobacco smoke (ETS) classified as a Group A (human) carcinogen.

1993/2001 Between 1993 and year-end 2001, the HOFFMANNs and their colleagues published five papers (116, 117, 118, 119, 120) dealing primarily with the toxic components in cigarette MSS as the cigarette was modified since the mid-1950s by inclusion of new design technologies to reduce its MSS yield and many of the toxic components in is particulate and vapor phases. Among the dozen or so PAHs listed as toxicants or tumorigens in MSS was B[*a*]A. Ranges of per cigarette yields listed for many such components were not only inconsistent among the five publications but occasionally were inconsistent between tables within a given publication [146, also see Table 2 in (43)]. The ranges for several of the PAHs, including B[*a*]A, obviously included per cigarette yields from the time when cigarettes delivered 35–40 mg of TPM. The MSS B[*a*]A yield was routinely listed as 20–70 ng/cig. When RODGMAN and GREEN (55) averaged the B[*a*]A data from several laboratories on the 1R4F Reference cigarette, a yield of 11.4 ng/cig was obtained. The MSS yields from the 1R4F cigarette are more representative of the modern cigarette than are the yields determined for commercial cigarettes manufactured in the 1960s and 1970s. Another fact not considered was the retention of inhaled TPM by the smoker which may vary from 50 to 90%. Smoking machines used in smoke component analysis do not exhale.

1994 Preliminary to its anticipated action against ETS, the Occupational Safety and Health Administration (OSHA) (147) prepared its own list of 43 significant tumorigenic components in tobacco smoke. Its list differed from that of HOFFMANN and HECHT (115). The OSHA list contained only 42 components. It appeared that ^{210}Po may have been inadvertently omitted. B[*a*]A was among the twelve PAHs listed by OSHA.

1997 SMITH *et al*. (148) published their assessment of nine "IARC Group I carcinogens" present in cigarette smoke. None of the nine was a PAH.

1999 In his discussion of tobacco smoke tumorigens and lung cancer, HECHT (149) listed eight PAHs as pulmonary carcinogens but did not include B[*a*]A among them. This was in contrast to his previous publication, co-authored with HOFFMANN (115), in which they listed B[*a*]A as one of the eleven tumorigenic MSS PAHs.

2000 RODGMAN *et al*. (150) discussed some of the cigarette design technologies that reduced the MSS PAH yield, including that of B[*a*]A, B[*a*]P, DB[*a,h*]A, and others. Some design technologies such as solvent extraction (SE) of tobacco or nitrate addition (NA) to tobacco while reducing MSS PAH yields introduced other problems such as increased per cigarette MSS yields of irritating aldehydes (SE), ketones (SE), and acids (SE), phenols (SE), and NNAs (SE, NA).

2000/2001 SMITH *et al*. (151) discussed the nine "IARC Group 2A carcinogens" present in cigarette smoke, three of which were PAHs: B[*a*]A, B[*a*]P, and DB[*a,h*]A. They also reviewed the inhibition of the tumorigenicity of these three PAHs by co-administration of non-tumorigenic PAHs (also present in cigarette MSS but at a much higher level than the three tumorigens). In the final publication on the IARC carcinogen groups, SMITH *et al.* (152) discussed the 48 "IARC Group 2B carcinogens" present in cigarette smoke, nine of which were PAHs.

2001 FOWLES and BATES (121), contrary to most of the recent lists of toxicants in tobacco smoke, still listed chrysene as one of the eleven PAH tumorigens despite the declassification of chrysene by IARC.

RODGMAN (8) reviewed the research conducted on PAHs in cigarette MSS: their identification, the precursors in tobacco of PAHs in MSS, technologies both successful and unsuccessful to decrease the per cigarette yield of PAHs, the failure by the 1964 ADVISORY COMMITTEE (127) to list over 85% of the PAHs identified in tobacco smoke by late 1963 [see Tables 2 and 4 in (8)] and its omission of over 92% of the literature references to PAHs in tobacco smoke [see Table 4 in (8)].

2002/2003 RODGMAN and GREEN (55) noted the seldom discussed issue of non-tumorigenic PAHs, present at a substantially higher level in MSS than the tumorigenic PAHs, and other MSS components that inhibit the tumorigenicity of the potently tumorigenic MSS PAHs, e.g., anthracene vs. B[*a*]A, B[*a*]A vs. B[*a*]P

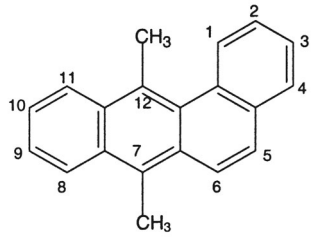
2003 RODGMAN (43) discussed the problems with the numerous lists of tumorigens in cigarette MSS and the texts accompanying the lists (114, 115, 116, 117, 118, 119, 120). The problems involved the following: 1) Inter- and intra-inconsistencies in the data tabulated in many of the lists, 2) the use of ranges of per cigarette yields for many MSS components, including the PAHs, based on analyses conducted from the mid-1955 to the publication date, 3) the equivalence of PAHs when no quantitative yield or only a single yield was reported for a PAH over more than four decades was considered equivalent to hundreds of analytical results for B[*a*]P, e.g., no per cigarette yield data were reported for dibenzo[*def,p*]chrysene (dibenzo[*a,l*]pyrene); a single value of 4 ng/cig was reported in 1960 by HOFFMANN and WYNDER (79) for DB[*a,h*]A, despite the fact that VAN DUUREN (153) had reported a yield of 5 ng/cig in 1958, and 4) the failure by most list authors to acknowledge the findings reported since the early 1950s that several non-tumorigenic PAHs (anthracene, phenanthrene, fluoranthene) or slightly tumorigenic PAHs (B[*a*]A) substantially decreased the tumorigenicity of several potent tumorigens such as B[*a*]P and/or DB[*a,h*]A when co-administered.

Seldom did the authors of such lists mention the human exposure to many of the PAHs in tobacco smoke that are also significant components of air

pollution and a variety of foodstuffs and beverages [see Table 5 in (43)].

2005 KALAITZOGLOU and SAMARA (154) described the distribution of PAHs between the particulate and vapor phases of the MSSs from a variety of cigarette types. B[*a*]A was quantified in the particulate phase of each cigarette type but was below the quantitation level in the vapor phase.

3 ANNOTATED CHRONOLOGY OF 7,12-DIMETHYLBENZ[*a*]ANTHRACENE (DMB[*a*]A)



Benz[*a*]anthracene, 7,12-dimethyl- [$C_{20}H_{16}$] [256]
CAS No. 57-97-6

Investigators who have studied and/or discussed the presence of DMB[*a*]A in tobacco smoke include: PIETZSCH (20, 21), SCASSELLATI-SFORZOLINI and MARIANI (22), WYNDER and HOFFMANN (77, 135), KRÖLLER (23, 24), STAMEY and DOBBINS (91), VAN DUUREN *et al.* (25), STAMEY *et al*. (155), RODGMAN (8, 156), and RODGMAN and GREEN (55).

In their catalogues of tobacco smoke PAHs, JOHNSTONE and PLIMMER (106), PHILIP MORRIS (107), ELMENHORST and RECKZEH (108), STEDMAN (109), and ISHIGURO and SUGAWARA (110) listed DMB[*a*]A as a component:

1938 BACHMANN *et al*. (157) presented the first report of the tumorigenicity of DMB[*a*]A to mouse skin. They were also the first to report the tumorigenicity of 7,8,12-trimethylB[*a*]A.

1939 MIKHAILOV and CHERNOVA (158) synthesized DMB[*a*]A from 7-methylbenz[*a*]anthracen-12-one by the Reformatsky reaction.

1951 HARTWELL in his compendium (31) cited 33 reports on laboratory studies conducted from 1938 through 1947 on the tumorigenicity of DMB[*a*]A. Almost without exception, in each study involving skin painting or subcutaneous injection of DMB[*a*]A, the administration induced tumors in the laboratory animals.

1953/1955 In his review of the relationship between electronic configuration and tumorigenicity, COULSON (129) listed the carcinogenicity of DBM[*a*]A to mouse skin as ++++ and its sarcogenicity on subcutaneous injection as +++ despite noting that calculations of the total K region electronic charge according to the PULLMAN and PULLMAN theory (130) for B[*a*]A and many of its multimethyl derivatives were "far from being satisfactory".

1956 KLEIN (159) demonstrated that as little as 1 ng of DMB[*a*]A may act as an initiator in mouse skin-painting assays. In citing this study in their 1964 review, WYNDER and HOFFMANN (77) noted:

> Because of the known effect of even the most minute amounts of a tumor initiator [M. KLEIN (159)] when followed by application of a tumor promoter, the contribution of polynuclear aromatic hydrocarbons in tobacco carcinogenesis cannot be given a numerical value.

1957 SHUBIK and HARTWELL (32) listed 61 bioassays conducted on the tumorigenicity of DMB[*a*]A between 1948 and 1953. As reported above, in almost every instance a large percentage of the treated animals developed tumors.

1958 DELLA PORTA *et al.* (160) reported that repeated intratracheal instillation of a colloidal suspension of DMB[*a*]A in gelatin induced tumors of the lung and bronchi in Syrian golden hamsters.

SHUBIK *et al.* (161) reported the significant inhibition of the carcinogenicity of DMB[*a*]A to mouse skin by the simultaneous administration of urethane (ethyl carbamate) and DMB[*a*]A. Two decades later, ethyl carbamate was identified as a component of MSS (162) and subsequently was listed repeatedly as a significant tumorigen in cigarette smoke (27, 114, 115, 116, 117, 118, 119, 120, 121).

1959 SALLEY and KRESHOVER (163) investigated the susceptibility of hamster cheek pouches to DMB[*a*]A and B[*a*]P. They indicated that relatively high doses of these PAHs are needed for carcinoma to develop in that area.

PIETZSCH (20) reported the identification of DMB[*a*]A in cigarette MSS, a finding that was criticized by COOK (164) and several times by WYNDER and HOFFMANN (77) who stated:

> The formation of a dialkylated benz[*a*]anthracene during pyrolysis appears questionable . . .

(cf. more recent data from SNOOK *et al.* (17, 18, 19). JOHNSTONE and PLIMMER (106) in their review on the composition of tobacco and tobacco smoke listed DMB[*a*]A as a component of tobacco smoke.

1960 RODGMAN and COOK (10) reported confirmation [see BONNET and NEUKOMM (66, 67)] of the presence of 5-methylbenz[*a*]anthracene in CSC. In the same study, RODGMAN and COOK identified several other alkylated PAHs (1-methyl-3,4-dihydronaphthalene, 1-methylpyrene, 2-methylpyrene, 4-methylpyrene) and partially identified several dimethylphenanthrenes and methylchrysenes, a methyl-B[*a*]P, and a methylfluoranthene. Subsequently, all of these alkylated PAHs in tobacco smoke were more completely defined in the mid-1970s by SNOOK *et al.* (17, 18, 19) with improved analytical technology.

1962 WYNDER and HOFFMANN (165) demonstrated the tumor-promoting property of tobacco smoke. A dose of 300 µg of DMB[*a*]A was used as the single initiator, followed by application three times weekly of a CSC solution in as low a concentration as 10%.

1964 WYNDER and HOFFMANN (77) questioned the report by PIETZSCH (20) on the identification of DMB[*a*]A in cigarette MSS. They stated:

> The formation of a dialkylated benz[*a*]anthracene during pyrolysis appears questionable . . .

This comment should be compared with the identification of the numerous multialkyl-B[*a*]As by SNOOK *et al*. who reported dimethyl-B[*a*]As in Peaks 99 through 102 in (17), in Peaks 26, 27, and 30 in (18), and in Peaks 129–134 in (19). In the latter study, two isomers were detected in each of Peaks 130 through 133. WYNDER and HOFFMANN also expressed dissatisfaction with data provided for identification of six alkyl-naphthalenes reported by JOHNSTONE and QUAN (166). They appeared unwilling to accept the presence of alkylated PAHs in pyrolysates of tobacco components or in tobacco smoke. However, WYNDER and HOFFMANN (77) did not question the 1963 reports by GROSSMAN *et al.* (167) on the pyrogenesis of alkylnaphthalenes during the pyrolysis of the tobacco isoprenoid solanesol. Subsequent research demonstrated the presence of numerous dialkyl-, trialkyl-, and tetraalkyl-B[*a*]As, all nine possible dimethylnaphthalenes, plus a great variety of other alkylated PAHs in cigarette MSS [see SNOOK *et al.* (17, 18,19)].

HANSCH and FUJITA (136) in their study of the correlation of biological activity and chemical structure listed the Log P of DMB[*a*]A. However, they did not define its tumorigenicity despite the fact that in their 1957 catalogue SHUBIK and HARTWELL (32) listed over 60 publications describing the potent tumorigenicity of DMB[*a*]A.

1966 VAN DUUREN *et al.* (25) reported the promoting action of CSC on mice treated with 150 μg of the initiator DMB[*a*]A. Other CSC PAHs reported to be potent initiators were benz[*e*]acephenanthrylene, B[*a*]P, and chrysene.

1967 WYNDER and HOFFMANN (135) reiterated their previous doubt not only about the presence of a dialkyl-B[*a*]A such as DMB[*a*]A in cigarette MSS reported by PIETZSCH (20) but also the formation of a dialkyl-B[*a*]A such as DMB[*a*]A during the pyrolyses reported by KRÖLLER (23, 24).

1968 STEDMAN (109) in his review of tobacco and tobacco smoke composition listed DMB[*a*]A as a PAH component of tobacco smoke.

WHEATLEY (168) reported studies in which the yield in rats of mammary tumors induced by treatment with DMB[*a*]A was decreased by treatment of the animals with various PAHs including the potent tumorigen 1,2-dihydro-3-methylbenz[*j*]aceanthrylene (3-methylcholanthrene).

1971 VAN DUUREN *et al*. (169) treated a group of 60 ICR/Ha Swiss mice once only with 50 μg of DMB[*a*]A, followed by repeated applications of CSC (40 mg in 0.1 mL of acetone; 5 paintings/week): 14 animals developed squamous cell carcinoma of the skin. The experiment was terminated after 573 days. In other experiments in the same series, the same dose of CSC alone induced skin cancer in only four animals. The dosage (50 μg) of DMB[*a*]A administered alone did not produce any skin carcinomas. Note: With an estimate of a per cigarette MSS yield of 5 ng of DMB[*a*]A, the level of DMB[*a*]A used (50 μg) was 10000 times the per cigarette MSS yield. In the 1R4F MSS, the B[*a*]P yields from three laboratories [see Table 1 in (55)] averaged 5.2 ng [see Table 2 in (43)]. The MSS level of B[*a*]P obviously always exceeds that of DMB[*a*]A since, to date, no one has determined the MSS yield of DMB[*a*]A.

1974 KOBAYASHI *et al.* (170) conducted an experiment similar to that described by VAN DUUREN *et al*. (169). They used DMB[*a*]A as an initiator in the study of the tumorigenicity to mouse skin of CSC.

1976 In their studies of tobacco smoke and tobacco extracts, BOCK and TSO (171) pretreated mouse skin with 125 μg of DMB[*a*]A and found that the tumor-promoting activity of tobacco extracts required the simultaneous application of two agents: One of high molecular weight, insoluble in organic solvents; the other of low molecular weight, soluble in organic solvents. They suggested the low-molecular weight compound could be nicotine.

DIPPLE (172), in his first detailed review of PAHs, listed DMB[*a*]A as highly tumorigenic, a classification repeated by DIPPLE *et al*. (28) in a second review in 1984.

SNOOK *et al.* (17, 18, 19) provided further data to refute the views of WYNDER and HOFFMANN (77, 135) that the presence of dialkylated (and presumably other alkylated) B[*a*]As in cigarette smoke was questionable. SNOOK *et al.* described the isolation and identification of methyl-B[*a*]As (Peaks 94–97) [cf. BONNET and NEUKOMM (66, 67), RODGMAN and COOK (10)], dimethyl-B[*a*]As (Peaks 99-102) [cf. PIETZSCH (20)], and trimethyl-B[*a*]As (Peaks 102 and 105) from CSC.

1977 SNOOK *et al.* (18) provided additional evidence for the presence of alkyl-B[*a*]As in cigarette smoke condensate with their identification of methyl-B[*a*]As (Peaks 22, 24, 25) and dimethyl-B[*a*]As (Peaks 26, 27, 30) in their chromatograms.

THE ROYAL COLLEGE OF PHYSICIANS (173) in its third report on smoking and health characterized PAHs and NNAs in tobacco smoke as the two chief classes of initiators of cancer.

1978 In their detailed study of alkylated PAHs in cigarette smoke condensate, SNOOK *et al*. (19) reported the identification of at least two different methyl-B[*a*]As (Peaks 123–127), at least two different dimethyl-B[*a*]As (Peaks 127, 129–134), at least two different trimethyl-B[*a*]As (Peaks 130, 131, 133, 134–136), and tetramethyl-B[*a*]As (Peaks 134, 137). These findings answer the question raised by WYNDER and HOFFMANN (77, 135) on the presence of dialkylated B[*a*]As in CSC.

1979 The 1979 report of the U.S. Surgeon General (111) did not mention DMB[*a*]A as a tumorigenic tobacco smoke component.

RINKUS *et al.* (142) reported that several cigarette smoke components, including DMB[*a*]A, were mutagenic in the Ames test (*Salmonella typhimurium*).

1980 BARTSCH *et al.* (143) reported that several cigarette smoke components, including DMB[*a*]A, were mutagenic in the Ames test (*Salmonella typhimurium*). They also reported on the mutagenicity of several of its metabolites.

1984 In their chapter in the two-volume, 1400+-page SEARLE-edited book (174), DIPPLE *et al*. (28) listed DMB[*a*]A as highly tumorigenic. It is interesting to note throughout this two-volume, 1400+- page book on

chemical carcinogenesis, the role of PAHs in general or any PAH in particular in the carcinogenicity of tobacco smoke was never discussed despite the hundreds of articles published on PAHs in tobacco smoke between the mid-1950s and 1984! The only smoke components discussed in terms of the tumorigenicity of tobacco smoke were the NNAs [see pp. 839–842 in (174)].

1985/1986 IARC (27) did not list DMB[*a*]A as a biologically active agent present in non-filtered cigarette MSS.

1990 HOFFMANN and HECHT (115) compiled a list of 43 "tumorigenic components of tobacco and tobacco smoke" but did not include DMB[*a*]A in their list. They also stated that DMB[*a*]A was:

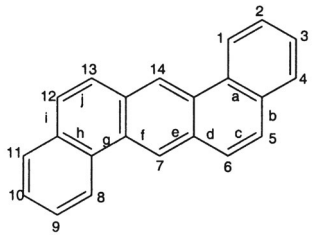
> . . . a highly tumorigenic, synthetic PAH which does not occur in tobacco smoke . . .

1993/2001 In none of the various publications by the HOFFMANNs and their colleagues (116–120) in which lists of tumorigenic PAHs were presented was DMB[*a*]A included as a tumorigenic PAH in cigarette MSS.

1997/2001 Because the IARC had not listed DMB[*a*]A as a Group I, 2A, or 2B carcinogen, it was not considered in the SMITH *et al.* reports (148, 151, 152) on these three classes of tumorigens in tobacco smoke.

4 ANNOTATED CHRONOLOGY OF DIBENZ[*a,h*]ANTHRACENE (DB[*a,h*]A)

The IUPAC numbering and lettering of DB[*a.h*]A and anthracene are as follows:



Dibenz[*a,h*]anthracene [$C_{22}H_{14}$] [278], CAS No. 53-70-3

Investigators who have studied and/or discussed the presence of DB[*a,h*]A in tobacco smoke include: WYNDER (83, 175), PIETZSCH (20, 21), VAN DUUREN and NELSON (176), WYNDER *et al.* (73), WYNDER and HOFFMANN (15, 77), HOFFMANN and WYNDER (78, 79), RODGMAN and COOK (10, 81), SCASSELLATI-SFORZOLINI and MARIANI (22), WALKER (177), KRÖLLER (23), RODGMAN (65), BADGER *et al.* (137), GUVERNATOR *et al.* (88), ROBB *et al.* (178), SNOOK *et al.* (18), MARRIOTT and WEAVING (179), GUERIN *et al.* (103), HECHT (149), RODGMAN *et al.* (150), and SMITH *et al.* (151). The following are catalogues of tobacco and/or tobacco smoke components that included DB[*a,h*]A: ROBERTS *et al.* (104), BENTLEY and BERRY (105), JOHNSTONE and PLIMMER (106), PHILIP MORRIS (107), ELMENHORST and RECKZEH (108), STEDMAN (109), and ISHIGURO and SUGAWARA (110). The following includes articles in which various smoke components, including DB[*a,h*]A, are listed and/or discussed as significant toxicants: USPHS (111, 112, 113, 127), IARC (27), HOFFMANN and WYNDER (114), HOFFMANN and HECHT (115), HOFFMANN *et al.* (116, 117), HOFFMANN and HOFFMANN (118, 119, 120), FOWLES and BATES (121), RODGMAN and GREEN (55), and RODGMAN (43):

1929 CLAR (180) and FIESER and DIETZ (124) independently reported the synthesis of DB[*a,h*]A .

1930 KENNAWAY and HIEGER (29) reported that DB[*a,h*]A was highly tumorigenic to mouse skin.

1932 DB[*a,h*]A was recognized as being structurally similar to B[*a*]P (44), first identified as a coal-tar constituent and also shown to be tumorigenic to mouse skin.

1937 BACHMANN (181) described a third synthesis of DB[*a,h*]A.

1938 DOBROVOLSKAIA-ZAVADSKAIA (182) reported a threshold limit for DB[*a,h*]A in a study involving subcutaneous injection of mice with DB[*a,h*]A solutions of decreasing concentration.

Subcutaneous injection of mice with DB[*a,h*]A			
DB[*a,h*]A, mg	No.	No. with tumors	TBA [a], %
0.01	328	37	11
0.005	364	13	4
0.0025	167	2	1
0.00125	158	0	0

[a] TBA = tumor bearing animals.

Similar findings were reported for other tumorigenic PAHs. For many years, such results were interpreted as indicating a threshold limit value.

1939 To test the carcinogenicity of DB[*a,h*]A to mouse stomach, MAGNUS (183) force fed 125 A strain mice [a] with a 0.4% solution of DB[*a,h*]A in olive oil. The mice began to die from lung adenoma. Of the 63 mice which survived the force feeding phase of the experiment (20 months), 95.2% died with lung tumors, 75% of which were malignant. The DB[*a,h*]A feeding not only increased the incidence of benign bronchial papilloma but also doubled the incidence of malignant tumors. However, SHIMKIN (184) noted:

> After subcutaneous or oral administration of large amounts of DB[*a,h*]A, absorption spectrum analysis fails to reveal the presence of the compound or of its derivatives in the lungs, although multiple tumors are induced.
>
> [a] The A strain mouse was specifically bred to develop lung adenoma spontaneously: 70 to 90% of untreated A strain mice will eventually die of lung adenoma.

1948 PFEIFFER and ALLEN (185) fed, skin painted, or injected intravenously 50 Rhesus monkeys with DB[*a,h*]A in an experiment whose duration was 10 years. No malignant tumors were produced in any of the animals.

1951 STEINER and FALK (128) reported that B[*a*]A[a], a weak carcinogen, inhibited the tumorigenicity of DB[*a,h*]A[a] when the two PAHs were administered simultaneously by subcutaneous injection.

> [a] Subsequently identified as a tobacco smoke component.

Indicative of the use of DB[*a,h*]A in tumorigenesis studies was the number cited by HARTWELL (31) that were conducted from 1930 through 1947: 239 in his main Table plus two more in his Addendum. Administration by a variety of routes resulted in a large %TBA in almost every study.

1956 TEBBENS *et al.* (186) found DB[*a,h*]A, a potent mouse-skin tumorigen, as a product of incomplete combustion of solid, liquid, and gaseous fuels.

1957 SHUBIK and HARTWELL (32) listed 36 bioassays conducted on the tumorigenicity of DB[*a,h*]A between 1948 and 1953. As reported above, in almost every instance a large percentage of the treated animals developed tumors.

1958 VAN DUUREN (153) identified DB[*a,h*]A and other PAHs in mainstream CSC; he listed this PAH as a "potent carcinogen".

PIETZSCH (20) issued a preliminary report of his identification of various PAHs, including DB[*a,h*]A, in tobacco smoke.

The tricyclic lactone sclareolide is a flavorful component of Oriental tobacco; it was first identified in tobacco by SCHUMACHER (187). Theoretically, sclareolide might yield DB[*a,h*]A on pyrolysis [see Figure 2 in (188)]. RODGMAN and COOK (189) demonstrated that addition of substantial quantities of sclareolide to a tobacco blend did not result in increased levels of DB[*a,h*]A in the CSC derived from the sclareolide-treated tobacco.

WYNDER *et al.* (73) identified several PAHs including DB[*a,h*]A in the pyrolysate (various temperature from 560 to 880 ˚C, in air, or in nitrogen) of a base-free hot hexane extract of tobacco and in CSC.

1959 PIETZSCH (20) published the detailed description of his identification of PAHs, including DB[*a,h*]A, in tobacco smoke.

JOHNSTONE and PLIMMER (106) in their review on the composition of tobacco and tobacco smoke listed DB[*a,h*]A as a PAH in tobacco smoke.

1960 HOFFMANN and WYNDER (78, 79) reported their identification of DB[*a,h*]A in CSC.

RODGMAN and COOK (10) reported the isolation of DB[*a,h*]A from CSC and its identification based on the agreement of the UV absorption spectra of the isolate and its oxidation product, the quinone 7,14-dibenz[*a,h*]anthracenedione, with those of authentic samples.

1963 DB[*a,h*]A was characterized by WYNDER and HOFFMANN (134) as a PAH with high carcinogenic activity. They noted that it had been previously identified in cigarette MSS.

1964 From his studies of a tobacco pyrolysate (700 ˚C) and a tobacco smoke condensate, KRÖLLER (23) reported the identification of DB[*a,h*]A as well as the identifications of phenanthrene, 4*H*-cyclopenta[*def*]phenanthrene (4,5-methylenephenanthrene), fluoranthene, B[*a*]P, DMB[*a*]A, and 1,2-dihydro-3-methylbenz[*j*]aceanthrylene (3-methylcholanthrene) in both. His identifications of DMB[*a*]A and 1,2-dihydro-3-methylbenz[*j*]aceanthrylene were questioned by WYNDER and HOFFMANN (135).

1965 ROBB *et al.* (178) described their method for a gas chromatographic analysis of the PAHs in CSC. In addition to B[*a*]P and several other PAHs (naphthalene, fluorene, anthracene, methylanthracene, phenanthrene, pyrene, 1-methylpyrene, fluoranthene, B[*a*]P, B[*e*]P, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, perylene, and benzo[*ghi*]perylene), they reported the presence of DB[*a,h*]A.

1968 STEDMAN (109) in his review of the composition of tobacco and tobacco smoke listed DB[*a,h*]A as a PAH component of tobacco smoke.

1977 THE ROYAL COLLEGE OF PHYSICIANS (173) characterized the PAHs and the NNAs as the two chief classes of initiators of cancer in tobacco smoke.

MARRIOTT and WEAVING (179) described a procedure for determining the per cigarette yield of DB[*a,h*]A in the condensates from tobacco and tobacco substitute smoking materials.

1981 EPA (190) listed DB[*a,h*]A as a carcinogen.

1982 IARC (191) found the evidence sufficient to classify DB[*a,h*]A as carcinogenic to laboratory animals.

THE NATIONAL TECHNICAL INFORMATION SERVICE (NTIS) (192) in its 3rd Annual Report on Carcinogens found sufficient evidence to consider DB[*a,h*]A carcinogenic to laboratory animals. In its report it was stated:

> Dibenz[*a,h*]anthracene and benzo[*a*]pyrene appeared to be equally effective . . . In a dose response study . . . on subcutaneous carcinogenicity with dibenz[*a,h*]-anthracene, benzo[*a*]pyrene, and 3-methylcholanthrene, dibenz[*a,h*]anthracene was shown to be effective at a lower dose than that effective for benzo[*a*]pyrene or 3-methylcholanthrene; its latent period was longer . . . The highest concentrations of dibenz[*a,h*]anthracene have been reported in airborne soot and in coal tar containing 64 to 705 mg/1000 m^3 and 230 milligrams per kilogram, respectively.

1985/1986 IARC (27) listed DB[*a,h*]A as a biologically active agent present in non-filtered cigarette MSS and listed an MSS yield of 4 ng/cig, that reported by HOFFMANN and WYNDER (78, 79). Overlooked by the IARC was the MSS yield of 5 ng/cig of DB[*a,h*]A reported by VAN DUUREN (153) in 1958. In its tabulation on the evaluation of the carcinogenicity of various tobacco smoke components, IARC listed the degree of evidence for the carcinogenicity of DB[*a,h*]A in animals as "Sufficient evidence". No specific comment was included on the degree of evidence concerning the carcinogenicity of this PAH in humans.

1986 HOFFMANN and WYNDER (114) in their tabulation of tumorigens in tobacco smoke and tobacco listed DB[*a,h*]A as a carcinogen with an incorrect per cigarette MSS yield of 40 ng/cig. The IARC had listed the MSS yield at 4 ng/cig. Subsequent lists by HOFFMANN *et al.* (115, 116, 117, 118, 119, 120) used the value 4 ng/cig.

1988 In his review of PAHs in foodstuffs, MAGA (50) reported the presence of DB[*a,h*]A in a variety of foodstuffs, e.g., barley malt, commercially broiled or fried hamburgers.

1990 HOFFMANN and HECHT (115) used the 1986 IARC report (27) to prepare a list of 43 significant "tumorigens" in tobacco and tobacco smoke: DB[*a,h*]A was included in their list. This list was cited by the Surgeon General in his 1989 report (144) and subsequently used by EPA (145) in its attempt to have ETS classified as a Group A (human) carcinogen.

1993/2001 In the publications by HOFFMANN *et al.* (116, 117), HOFFMANN and HOFFMANN (118, 119, 120) and FOWLES and BATES (121), DB[*a,h*]A was included as a tumorigenic component of cigarette MSS. In each publication the yield of DB[*a,h*]A was listed as 4 ng/cig, again neglecting to include the report by VAN DUUREN (153) of 5 ng/cig of DB[*a,h*]A.

1994 OSHA (147) prepared its own list of 43 significant tumorigenic components in tobacco smoke. Its list differed from that of HOFFMANN and HECHT (115). The OSHA list contained only 42 components. ^{210}Po may have been omitted. DB[*a,h*]A was among the twelve PAHs listed.

1999 In his list of eight tumorigenic PAHs in cigarette MSS, HECHT (149) listed DB[*a,h*]A at a yield of 4 ng/cig. HECHT rated DB[*a,h*]A as a "substantially stronger lung tumorigen" than B[*a*]P but noted that its yield (4 ng/cig) is much lower in cigarette MSS than that of B[*a*]P (20–40 ng/cig), a range that includes data from cigarette MSS analyzed three to four decades earlier.

2000 SMITH *et al*. (151) discussed the nine "IARC Group 2A carcinogens" present in cigarette smoke, three of which are PAHs, including DB[*a,h*]A. From their tabulation of the various reports on MSS DB[*a,h*]A yield, they listed a range of 4 to 76 ng/cig. In their brief outline of DB[*a,h*]A, SMITH *et al*. noted exposures to it (other than tobacco smoke) included foodstuffs and air pollutants. It is among the five PAHs found in heated foodstuffs (192, 193).

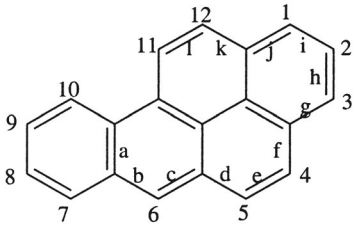
2002/2003 When RODGMAN and GREEN (55) averaged the DB[*a,h*]A data from several laboratories on the 1R4F Reference cigarette, a yield of 0.4 ng/cig was obtained. The MSS yields from the 1R4F cigarette are more representative of the modern cigarette than are the yields determined for commercial cigarettes in the late 1950s, the 1960s, and the 1970s.

2003 In a discussion of the inconsistencies in the various lists of tumorigenic PAHs (116, 117, 118, 119, 120, 121), RODGMAN (43) noted: 1) the failure to include published data on the per cigarette MSS DB[*a,h*]A yield and 2) the use of a per cigarette MSS yield from a cigarette sold several decades before the lists were assembled.

2005 KALAITZOGLOU and SAMARA (154) described the distribution of PAHs between the particulate and vapor phases of the MSSs from a variety of cigarette types. DB[*a,h*]A was quantified in the particulate phase of each cigarette type but was not detected in the vapor phase.

5 ANNOTATED CHRONOLOGY OF: BENZO[*a*]PYRENE (B[*a*]P)

The IUPAC lettering of the pyrene molecule and numbering of the B[*a*]P molecule are as a follows:



Benzo[*a*]pyrene [$C_{20}H_{12}$] [252], CAS No. 50-32-8

Despite the numerous statements since the mid-1950s that the level of B[*a*]P in CSC accounts for very little (<3%) of the observed tumorigenicity of the CSC used in mouse skin-painting studies, numerous analytical methodologies have been investigated and proposed to improve the quantitation of B[*a*]P in CSC from the early 1960s to the present. The following is a chronological list of such studies from the early 1960s to date: BARKEMEYER (194), TESTA *et al*. (195), ROBB *et al*. (178), SCHMELTZ *et al*. (196), OLIVER (197), PAILER (198), STAMEY and DOBBINS (91), DAVIS *et al*. (199), HEGE (200), STAMEY *et al*. (155), OAKLEY *et al*. (201), ALLEN (202), KLIMISCH and AMBROSIUS (203), KLIMISCH and KIRCHHEIM (96), SHELAR *et al*. (204), RISNER (100, 101, 102, 205), RISNER and CONNOR (206), DUMONT *et al*. (207), FROST *et al*. (208), DEMETRIOU and SCHEPERS (209), MOORE *et al*. (210), and C. ZHANG *et al*. (211).

The following is a list of catalogues of tobacco and/or tobacco smoke components that included B[*a*]P: ROBERTS *et al*. (104), BENTLEY and BERRY (105), JOHNSTONE and PLIMMER (106), PHILIP MORRIS (107), ELMENHORST and RECKZEH (108), STEDMAN (109), and ISHIGURO and SUGAWARA (110).

The following includes articles in which various smoke components, including B[*a*]P, are listed and/or discussed as significant toxicants: USPHS (111, 112, 113), IARC (27), HOFFMANN and WYNDER (114), HOFFMANN and HECHT (115), HOFFMANN *et al*. (116, 117), HOFFMANN and HOFFMANN (118, 119, 120), FOWLES and BATES (121), RODGMAN and GREEN (55), and RODGMAN (43):

1924 Classical study by KENNAWAY (212) demonstrated that isoprene pyrolysis yields a "tar" of considerable carcinogenicity to the skin of laboratory animals. "Tars" similar to those generated by KENNAWAY were subsequently shown to contain PAHs whose UV spectra were similar to but not identical with that of B[*a*]A.

1932/1933 COOK *et al*. (44) isolated several PAHs by the fractionation of two tons of coal tar. Among them was a new, potent mouse-skin carcinogen which COOK *et al*. demonstrated, by synthesis, to be B[*a*]P. B[*a*]P induced carcinomas at the site of skin painting with a B[*a*]P solution (30). It was the second individual chemical compound to do so, the first being DB[*a,h*]A (29). This discovery eventually was considered a milestone in cancer research because it led to the eventual synthesis of hundreds of PAHs and their derivatives and the beginning of extensive research on chemical carcinogenesis. By the late 1940s, the multitude of PAHs carcinogenic to mouse skin or sarcogenic on subcutaneous injection triggered a sequence of theories advanced to correlate the structure of the PAHs and their degree of tumorigenicity. Among them were the theories by COULSON (129), PULLMAN and PULLMAN (130), DAUDEL and DAUDEL (213), and FIESER (214). None of the early theories on the relationship between structure and tumorigenicity dealt with 1) the PAH diol, epoxide, or diol-epoxide metabolites and their role in the tumorigenicity of specific PAHs (28) or 2) the fact that in each situation – air pollutants, tars, heated foodstuffs, etc. – a given PAH was a component of a complex mixture and was never the only PAH present in the mixture.

1934 The findings of COOK *et al.* (44) on the presence and level of B[*a*]P in coal tar were confirmed by WINTERSTEIN and SCHÖN (215).

1939 ROFFO (6, 7, 216) reported the identification of a "benzopyrene-like" substance in the destructive distillate from tobacco. The UV spectrum of the substance was similar to that of B[*a*]P.

1946/1947 CRABTREE (217) reported that several nontumorigenic PAHs (naphthalene[a], anthracene[a], phenanthrene[a]) inhibited the tumorigenicity to mouse skin of the potent tumorigens B[*a*]P[a] and DB[*a,h*]A[a]. Other PAHs (phenanthrene[a], fluoranthene[a]), nontumorigenic to mouse skin, were later found to inhibit the tumorigenicity of DMB[*a*]A to mouse skin [see DIGIOVANNI *et al.* (218)].

[a] Subsequently, this PAH was identified in tobacco smoke.

BERENBLUM and SHUBIK (219), in their studies of cocarcinogenesis, described the potentiating effect of croton oil (which itself is almost always reported as noncarcinogenic) on the carcinogenicity of B[*a*]P to mouse skin.

IVERSEN (220) reported that, in a 33-year old male injected with 4 to 6 g of B[*a*]P, the metabolism of B[*a*]P in the human paralleled that in the mouse as reported by WEIGERT and MOTTRAM (221). Spectrophotometric studies revealed the same metabolites in both species. From their observations, WEIGERT and MOTTRAM proposed that B[*a*]P was chemically bonded to some component of a cell.

1947/1984 Over the next four decades, the proposal by WEIGERT and MOTTRAM was subjected to intense research to define: 1) the relationship not only between B[*a*]P and a cellular component (DNA) but also between several other PAHs and DNA and 2) the various diols, epoxides, and diol-epoxides generated during the metabolism of administered PAHs. In their review, DIPPLE *et al.* [see pp. 91–109 in (28)] summarized the research on PAH-DNA reactions as well as the relationship of the metabolic PAH diols, epoxides, and diol-epoxides to tumorigenesis. Among the 12 PAHs so discussed in 2) were B[*a*]A, DMB[*a*]A, DB[*a,h*]A, and of course, the more than a dozen metabolites of B[*a*]P. Among the 12 PAHs discussed by DIPPLE *et al.* were several that formed metabolites similar to those of B[*a*]P or DB[*a,h*]A but generally were and still are classified as nontumorigenic, e.g., B[*e*]P. chrysene, phenanthrene. In addition to providing references to their own contributions, DIPPLE *et al.* provided over 150 references to major contributions on the PAH metabolites, including contributions by such noted carcinogenesis investigators as BIGGERS, BOYLAND, C.S. COOPER, P. DAUDEL, LEVIN, and the MILLERS. The mutagenicity of the metabolites of B[*a*]P was reviewed by LEVIN *et al.* (222).

1948 PFEIFFER and ALLEN (185) fed, skin painted, or injected intravenously 50 Rhesus monkeys with B[*a*]P in an experiment with a duration of 10 years. No malignant tumors were produced in any of the primates by any of the administration routes studied.

1951 HARTWELL (31) listed the results of 337 studies conducted from 1932 through 1947 that involved bioassays of B[*a*]P. Tumorigenicity was observed in a large percentage of the treated animals no matter the administration route.

1953 COOPER and LINDSEY (223) reported the identification of several PAHs (anthracene, pyrene) in a CSC generated by a procedure simulating human smoking.

1954 COOPER *et al.* (224) reported the presence of B[*a*]P [supposedly confirmed by UV absorption spectrometry and fluorescence spectrography (see criticisms by FIESER (225) in 1957 of the use of incomplete UV spectra as basis for identification)] in chromatographic fractions from cigarette MSS: They estimated the level in MSS to be 10 ng/cig.

LEFEMINE *et al*. (226) reported the detection of B[*a*]P in a cigarette paper pyrolysate.

1955 In two separate publications, COOPER and LINDSEY reported B[*a*]P in the combustion products from cigarette paper (227) and in cigarette MSS (228). Their identification of B[*a*]P was based on UV analysis.

SEELKOPF (229) reported the presence of naphthalene, anthracene, and traces of B[*a*]P in cigarette "tar".

LAM (230) reported B[*a*]P (identified by UV spectrophotometry) in the pyrolysate of the saturated aliphatic hydrocarbons obtained by extraction from cigarette butts.

WYNDER and WRIGHT (231) and WRIGHT and WYNDER (232) estimated that there can be no more than one part per million (ppm) of B[*a*]P in the total mainstream CSC. They stated (231):

> . . . the concentration in which B[*a*]P seems to be in cigarette tar is insufficient to account for the observed carcinogenic activity to mouse epidermis.

STOCKS and CAMPBELL (233) calculated the amount of B[*a*]P inspired per year by a resident in an urban, a mixed urban-rural, and a rural area of England. The values obtained were 450, 157, and 41 μg, respectively. Contrast these values with the amount inspired by a pack-a-day inhaling smoker, e.g., 146 μg/year (with a value of 2 μg in the MSS of 100 cigarettes).

Other 1955 publications on PAHs, including B[*a*]P, in cigarette MSS included those of LETTRÉ and JAHN (61) and LINDSEY (234).

1955/1956 CARDON *et al.* (235) described the identification of B[*a*]P in the smoke from cigarette paper and tobacco. This was the first U.S. report of B[*a*]P in smoke generated from tobacco, cigarette paper, and cigarettes. Then ALVORD and CARDON (236) described a method to inhibit its formation, the addition of ammonium sulfamate. The UV spectrum used to identify B[*a*]P in the various smokes was deemed by FIESER (225) to be inadequate.

FALK and KOTIN (131) reported the per cigarette yields of B[*a*]P and B[*a*]A in MSS and SSS.

1956 RODGMAN (9) isolated B[*a*]P from the CSC from a non-filtered cigarette containing a commercial American tobacco blend. The B[*a*]P was identified unequivocally by mixture melting point analysis, correspondence of the UV and IR spectra of the isolate with those of an authentic sample, and mixture melting point analysis of the picrate of the isolate and an authentic sample of B[*a*]P picrate. This was the first reported instance of the isolation of B[*a*]P from CSC in crystalline form. In 1959, WYNDER and HOFFMANN subsequently reported the isolation of crystalline B[*a*]P from CSC (15).

LYONS (62) reported 7.4 μg B[*a*]P in the MSS from 500 cigarettes (14.8 ng/cig), through UV fluorescence spectrography and absorption spectrophotometry.

WYNDER and WRIGHT (237) reported B[*a*]P, detected by spectrographic analysis, to be present in the neutral fraction of CSC. They stated:

> . . . only the paper burned unrealistically in bulk shows the presence of B[*a*]P above the detectable level [1 to 10 ppm].

Examination of pyrolysis data from LAM (238) indicated that B[*a*]P was not a valid 'marker' or 'indicator' for PAHs with four or more rings generated during pyrolysis. Subsequently, this invalidity was shown also to be true for B[*a*]P generated during the tobacco smoking process; cf. the results of the NCI Tobacco Working Group (TWG) studies described by GORI (93) and NCI (139).

KURATSUNE (239) detected B[*a*]P in pyrolysates from various materials but *none* in cigarette MSS. Traces were detected in cigarette butts and ashes. He concluded:

> . . . small amounts of benzopyrene are produced by smoking, but then only a very small part . . . thus formed appears in the inhaled smoke.

In a continuation of the search for PAHs with emphasis on B[*a*]P in CSC, publications by the following investigators were issued in 1956: BABIN *et al.* (240), BONNET and NEUKOMM (66), CAMPBELL and LINDSEY (241), DICKEY and TOUEY (242), GILBERT and LINDSEY (68, 69), LETTRÉ *et al.* (243), and WYNDER (244).

1956/1957 WRIGHT (245) demonstrated that cigarette paper burned in a manner much like its cylindrical form in a cigarette did not yield B[*a*]P. Only cigarette paper burned unrealistically in bulk yielded B[*a*]P.

1957 Citing other investigators of carcinogenic PAHs in tobacco "tar": [COOPER and LINDSEY (223, 228), LYONS (62), LATARJET *et al.* (246), etc.], WYNDER and WRIGHT (237) stated in their 1957 publication:

> . . . we have demonstrated experimentally . . . that 0.0001 per cent or even 0.0005 per cent benzopyrene in acetone will not produce any tumors in the present experimental mouse or rabbit groups. Thus, there is conclusive proof that the animal results cannot be solely due to the benzopyrene content of tobacco [*smoke*].

According to WYNDER and WRIGHT:

> The benzpyrene content of the total tar as well as the active fractions is far too low to account alone for the positive results [in laboratory animal]. So far, no carcinogens have been identified in large enough quantity in tobacco tar or its fractions to account for the observed activity.

FIESER (225), the dean of U.S. organic chemists proficient in PAH chemistry, described the early studies to identify B[*a*]P in CSC as follows:

> The evidence, however, is not clear. British (228) and American (226, 235, 236) groups have claimed identification of benzpyrene, following extensive chromatography of tars from cigarette smoke, but in each case the evidence of identity is correspondence of the smoke factor with the synthetic carcinogen in fluorescence spectrum, coupled with correspondence of the two materials in *one region* of the ultraviolet absorption spectrum. The spectrum improves with increasing purification for a time and then becomes no better. In the absence of complete ultraviolet correspondence, the smoke-factor reported by the two groups of investigators, can be described as nothing more than a 'benzpyrene-like substance', which may or may not be carcinogenic.

FIESER (225) also reported that his two Harvard University colleagues, HUANG and JOHNSTON, were able to detect B[*a*]P in 20 g of CSC spiked with B[*a*]P (9.7 μg/g) but were unable to identify B[*a*]P in 20 g of unspiked CSC. FIESER also commented on the fact that KURATSUNE (239) did not identify B[*a*]P in tobacco smoke. KURATSUNE commented:

> The analysis of the smoke from 400 cigarettes . . . revealed only a minimal trace of (B[*a*]P) . . . the presence of a trace of (B[*a*]P) appears doubtful.

Later, KURATSUNE and HUEPER (247) reported B[*a*]P in a roasted coffee product.

NOTE: FIESER was the member of the 1964 Advisory Committee to the U.S. Surgeon General and responsible for the chapter on tobacco and smoke chemistry [see Chapter 6 on smoke chemistry in (127)]. Obviously in 1957, FIESER was unaware of the 1956 report by RODGMAN (9) in the isolation of crystalline B[*a*]P from MSS or the 1955–1956 reports by FALK and KOTIN (131) on the per cigarette yields of B[*a*]P in MSS and SSS. FIESER (248) eventually accepted the presence of B[*a*]P in CSC after the 1959 published report by WYNDER and HOFFMANN (15) of their isolation of crystalline B[*a*]P from CSC. It is interesting to note that FIESER (225) reported that his staff could not find B[*a*]P in CSC but could find it in an extract of roasted coffee beans.

WYNDER *et al.* (249) demonstrated in a mouse skin-painting study with various dose levels of B[*a*]P that there was a threshold limit value to the B[*a*]P dose. No tumorigenicity was observed in mice painted with a B[*a*]P solution containing the B[*a*]P level in the MSS from several hundred mid-1957 commercial cigarettes. From the results of their study, they noted:

> These data . . . again demonstrate that benzopyrene cannot be the only, and most likely the major, carcinogen in cigarette tar. It is of interest that both these fractions present spectrographic evidence of higher aromatic hydrocarbons in 50 times the concentration of benzopyrene.

Comments such as these were the basis for the search for the "supercarcinogen" in cigarette MSS, a search conducted by WRIGHT that after 18 months had failed to yield a PAH that could be classified as a "supercarcinogen".

Their result of a threshold limit confirmed the previously reported B[*a*]P finding of POEL (250) as well as subsequent findings by POEL *et al.* (251) and POEL and KAMMER (252). A similar threshold limit had been reported for DB[*a,h*]A by DOBROVOLSKAIA-ZAVADSKAIA (182).

SHUBIK and HARTWELL (32) listed 64 bioassays conducted on the tumorigenicity of B[*a*]P between 1947 and 1953. As reported above, in almost every instance a large percentage of the treated animals developed tumors. Thus, the two compendia (31, 32) listed a total of 401 studies on the tumorigenicity of B[*a*]P.

The number of bioassays demonstrating the potency of B[*a*]P as a tumorigen was the obvious reason that the number of reports on B[*a*]P and other PAHs in

cigarette MSS began to mount dramatically. During 1957, reports by the following investigators were also completed: AHLMANN (72), LYONS and JOHNSTON (70), NEUKOMM (253), NEUKOMM and BONNET (71), PIETZSCH (254), RAND *et al.* (255), RODGMAN (11, 12), WIESKE (256), and WYNDER (257).

1958 VAN DUUREN (258) reported that B[*a*]P possessed strong carcinogenic potential in his experiments with the PAHs in cigarette MSS. However, he concluded:

> . . . the carcinogenic hydrocarbons found so far do not by themselves appear to be present in sufficient concentrations to account for the observed activity.

WYNDER *et al.* (73) suggested the use of the level of B[*a*]P in CSC as a 'marker' or 'indicator' for the levels of other tumorigenic PAHs in CSC as well as for the specific tumorigenicity of the CSC to mouse skin.

BENTLEY and BURGAN (259) of Imperial Tobacco (U.K.) reported the identification of B[*a*]P in cigarette MSS and the effect on its MSS level of addition of various combustion modifying materials to the tobacco.

GELLHORN (260) concluded that CSC was cocarcinogenic or a promoter because application of a mixture of B[*a*]P + CSC to mouse skin resulted in neoplastic changes, whereas the same amount of B[*a*]P applied alone failed to elicit tumors.

1958/1959 RODGMAN [11, 13; also see Figure 1 and Table 3 in (8)] reported that removal of organic solvent-soluble components (saturated aliphatic hydrocarbons, phytosterols, terpenoids such as solanesol) from tobacco, followed by smoking of the extracted tobacco in cigarette form resulted in a decreased level of PAHs, including B[*a*]P, in the CSC. The extraction of various tobacco types and blends was performed by ASHBURN (261). Several patents based on these findings were issued (262). Extraction of tobacco with organic solvents to remove PAH precursors and lower PAHs in cigarette MSS was studied by others at that time, e.g., TENNESSEE EASTMAN (263), and WYNDER *et al*. (264). RODGMAN and COOK (14) showed that individually "spiking" of a tobacco blend with each of these organic solvent-soluble components (saturated aliphatic hydrocarbons, phytosterols, and solanesol) at double and triple the level in the tobacco blend resulted in CSCs with increased PAH contents and increased B[*a*]P contents, and the increases were proportional to the amount of the added component.

In 1979, SEVERSON *et al*. (265) confirmed the RODGMAN-COOK 1958 finding that solanesol is a much more significant tobacco precursor of PAHs in MSS than are the tobacco saturated aliphatic hydrocarbons.

1958 The 1958 publications noted above were accompanied by many other reports devoted to PAHs in CSC. Many emphasized the demonstration of the presence of B[*a*]P, its quantitation in cigarette MSS, and its tumorigenicity to mouse skin: BONNET (266), CARDON (267), CUZIN (268), HUBERT-HABART *et al*. (269), ORRIS *et al.* (270), RODGMAN and COOK (14), VAN DUUREN (153), VAN DUUREN and NELSON (176), SCASSELLATI-SFORZOLINI and SALUCCI (271), and WYNDER *et al.* (73).

1959 WYNDER and HOFFMANN (15) reported the isolation of crystalline B[*a*]P from cigarette MSS. They presented data which supported previously published data showing B[*a*]P to be a potent carcinogen to mouse skin. They wrote [see p. 1079 in (15)]:

> . . . several carcinogenic higher aromatic polycyclic hydrocarbons [are] present in tobacco smoke condensate. These include benzo[*a*]pyrene . . . , benzo[*e*]pyrene . . . , chrysene . . . , benz[*a*]anthracene . . . , dibenz[*a,h*]anthracene . . . , and dibenzo[*a,i*]pyrene . . . From the amount in which these materials have been found in tobacco smoke condensate it was evident that these, by themselves, could not account for the total biological activity observed.

Other 1959 reports on some aspect of PAHs in general and B[*a*]P in particular in cigarette MSS include those of BONNET and NEUKOMM (66, 272), BURGAN (273), DIKUN (274), DIKUN and CHUSHKIN (275), LINDSEY (74, 75), LINDSEY *et al*. (276), PIETZSCH (20), RODGMAN (277), SULA (278), and ZAPIOR *et al*. (279).

1960 BENTLEY and BURGAN (280) studied the inhibition of the formation of B[*a*]P in MSS by addition of various compounds to the tobacco. Their findings with regard to the inhibitory effect on B[*a*]P formation of nitrate addition resulted in considerable research on nitrate addition to tobacco blends during the next decade.

HOFFMANN and WYNDER (79) reported the per cigarette yields of B[*a*]A, B[*a*]P, and DB[*a,h*]A from mainstream CSC obtained by smoking 430 commercial American non-filtered cigarettes. Relative to a standard, it was estimated that a 35% to 40% loss occurred during the determination of the per cigarette yield of B[*a*]P. A yield of 23 ng/cig was reported. They also repeated the description of their previous isolation of crystalline B[*a*]P from 3200 cigarettes (15).

The Nobel Laureate SZENT-GYORGYI proposed the use of a filter-tip additive chloranil to reduce the PAHs in cigarette MSS (281). Chloranil forms a complex with most PAHs. Study of the proposal indicated that, because of the nature of the MSS aerosol, neither chloranil nor 2,4,7-trinitrofluorenone specifically reduced PAHs in MSS.

From examination of the research on PAHs in cigarette MSS in 1960, it is obvious from the number of journal publications and conference presentations that 1960 was a highly productive year. In addition to the 1960 reports summarized above, there were publications and/or presentations on the chemistry of MSS, primarily its PAH content, by CANDELI *et al.* (282), CLEMO (283), DRUCKREY *et al*. (284), HOFFMANN and WYNDER (78, 79), HUBERT-HABART (285), MOURON *et al.* (286), MUEL and LACROIX (287), PAVLU and SULA (288), RODGMAN and COOK (81), RODGMAN *et al*. (289), and TAKAYAMA and OOTA (290). All of these reports dealt with not only PAHs in cigarette MSS but also its B[*a*]P content. The biological properties of MSS relative to its B[*a*]P content were reported by CUZIN *et al.* (291) and DIKUN *et al*. (292). NEUKOMM and BONNET (293) received a patent on solvent extraction of tobacco to remove PAH precursors.

1961 The production of lung tumors following intratracheal administration of B[*a*]P was first demonstrated by RYAZANOV *et al.* (294) in a study in which one or five administrations of *100 mg* of B[*a*]P to eight rats produced at least three lung tumors.

According to BURNEY (295), citing data from U.S. Public Health Service surveys, the MSS from one pack

of cigarettes per day would contribute about 60 μg of B[*a*]P per year. A person inhaling air in some U.S. cities would take in from 110 to 150–160 μg of B[*a*]P per year.

From his experiments with B[*a*]P and CSC, DRUCKREY (296) reported that he found the amounts of B[*a*]P present in mainstream CSCs could not account for more than a few percent of the observed biological activity of the tobacco products. This result was similar to that reported by WYNDER and WRIGHT (237) and WYNDER and HOFFMANN (15).

WYNDER and HOFFMANN (297) wrote:

> The polynuclear aromatic hydrocarbons are mainly formed during the combustion of tobacco. The tobacco of our standard cigarettes contains only very minute quantities of benzo[*a*]pyrene (0.02 ppm). A bioassay indicates that these polycyclic hydrocarbons of the condensate by themselves, however, can account for not more than 3 per cent of the total biological activity.

1961/1962 WYNDER and HOFFMANN (165) (see also WYNDER and HOFFMANN [pp. 330–331 in (77), pp. 370–371 in (135)]) demonstrated that the saturated aliphatic hydrocarbon fraction in CSC substantially reduced the tumorigenicity of B[*a*]P to mouse skin. The components of the saturated hydrocarbon fraction ranged from C_{12} (dodecane) to at least C_{40} (tetracontane), each hydrocarbon being present as the *normal*, *iso*, and *anteiso* isomer. Their studies with B[*a*]P and two of the most plentiful components of this fraction, the *n*-C_{31} and *n*-C_{35} hydrocarbons, showed that these hydrocarbons, at ratios of saturated aliphatic hydrocarbon:B[*a*]P of 200:1 and 100:1, exerted a significant inhibiting effect on the specific tumorigenicity of B[*a*]P at both ratios for both saturated hydrocarbons. WYNDER and HOFFMANN also showed that enhancement of the level of the saturated aliphatic hydrocarbon fraction in CSC substantially reduced the specific tumorigenicity of the CSC in mouse skin-painting experiments.

1961 Not only were there several 1961 reports on PAHs, including B[*a*]P, in cigarette MSS by ALEXANDROV *et al*. (298), GRIMMER (299), GUERIN (300), MOURON *et al*. (301), RODGMAN (64), SCASSELLATI-SFORZOLINI and MARIANI (302), and SCASSELLATI-SFORZOLINI and SALDI (303), but also there were reports on the effect of organic solvent-extraction of tobacco on the level of toxic components, including PAH yields in MSS, by MATHEY (304) and SASMOCO (305).

1962 BARKEMEYER (194), BENTLEY (306), DIKUN *et al.* (307), and LINDSEY (308) reported on their studies on PAHs and the yield of B[*a*]P in cigarette MSS.

1962/1963 ROE (309) conducted a study in which he investigated the role of B[*a*]P as a possible initiator in the carcinogenic effect of cigarette MSS toward mouse skin. From his experiments with B[*a*]P and CSCs at various dose levels, he reported that the B[*a*]P level of CSC could be increased 10-fold without a significant increase in tumor yield. He interpreted these results as indicating that B[*a*]P is not necessarily of primary importance in the initiation of skin tumors by CSC [cf. LAZAR *et al.* (310) and HOFFMANN and WYNDER (138) below]. ROE also reported that repeated application for 68 weeks of 1.25 μg of B[*a*]P in acetone to skin of mice failed to produce tumors.

1963 WYNDER and HOFFMANN (134) wrote:

> Benzo(a)pyren und Phenole erwiesen sich als geeignete 'Indikatoren' für die kanzerogene Aktivität des Testmaterials. *Translation*: Benzo[*a*]pyrene and phenol were demonstrated to be suitable 'indicators' for the carcinogenic activity of the test materials [various cigarette smoke condensates].

They discussed possible means to reduce PAH yield in and the tumorigenicity of MSS by the use of compounds such as nickel acetate or cupric nitrate added to the tobacco, by selection of tobaccos, and by filtration systems that removed selected components from the MSS. Once again they noted:

> Biostatistische Berechnungen ergaben, dass die Tumoraktivität von Zigarettenrauchkondensat nur zu etwa drei Prozent durch die Anwesenheit mehrkerniger Kohlenwasserstoffe erklärt werden kann. *Translation*: Biostatistical calculation showed that only about three percent of the tumorigenicity of cigarette smoke condensate can be explained by the presence of the polycyclic hydrocarbons.

HOFFMANN and WYNDER (311) reported that B[*a*]A reduced the carcinogenic activity of B[*a*]P in a mouse skin-painting study. In 1951, STEINER and FALK (128) had shown a similar inhibition of B[*a*]A for the sarcoma generation of the potent sarcogens DB[*a,h*]A and 1,2-dihydro-3-methylbenz[*j*]aceanthrylene on subcutaneous injection.

Other 1963 reports pertinent to PAHs in MSS were published by DRUCKREY and SCHILDBACK (312), GILAV and SHABTAI (313), HOFFMANN *et al*. (314), NEURATH and HORSTMANN (315), SCHERBACK *et al.* (316), and WYNDER and HOFFMANN (134). The effect of tobacco treatment (organic solvent extraction, etc.) on MSS yield of PAHs, including B[*a*]P, was reported by CUZIN *et al.* (317), NEUKOMM and BONNET (71, 318), and WALTZ and HÄUSERMANN (319).

1964 The ADVISORY COMMITTEE in its report (127) to the U.S. Surgeon General reported that B[*a*]P was "one of the most potent of all the carcinogens now known". The Committee further noted that, along with dibenzo[*a,i*]pyrene, "benzo[*a*]pyrene is one the two most potent of the seven carcinogens detected in tobacco smoke and it is present in much larger quantity than any of the other carcinogens listed".

The Committee also commented that DRUCKREY (296) and WYNDER (83) had emphasized that:

> . . . the benzo[*a*]pyrene concentration of various tobacco and smoke preparations is only sufficient to account for a very small part of the carcinogenicity of these materials.

FIESER (248), in his acceptance of the presence of B[*a*]P in cigarette MSS after the report of its isolation in crystalline form from CSC by WYNDER and HOFFMANN (15), postulated the precursor in tobacco of B[*a*]P in MSS:

> The hydrocarbon (B[*a*]P) is not present in tobacco but is formed by pyrolysis of cellulose and other constituents of tobacco at the high temperature of the burning cigarette . . . the mechanism by which cellulose, on pyrolysis, yields benzpyrene is still a mystery. However, it is clear that benzpyrene cannot be eliminated from smoke by any conceivable method of processing tobacco (since it is not present in tobacco) . . .

This postulate was advanced despite the wealth of information in the pre-1964 literature that pyrolysis of biopolymers such as cellulose and starch did yield PAHs, including B[*a*]P (320). However, the major contributors to PAHs in MSS were the hexane-soluble components that constituted about 10% of the tobacco weight and included the phytosterols [WYNDER *et al*. (73), see last-paragraph addition to (264)], terpenoids such as solanesol, and the saturated aliphatic hydrocarbons (137, 230, 238, 321).

WYNDER and HOFFMANN (77) stated:

> Benzo[*a*]pyrene and phenol have been chosen as first 'chemical indicators' for the activity of smoke condensate. They indicate the concentration of carcinogenic PAH as a whole with benzo[*a*]pyrene and tumor promotion of the phenol group with phenol itself . . . One should . . . attempt to establish chemical indicators on the basis of which the tumorigenic activity of a given 'tar' could be predicted. In this manner we regard benzo[*a*]pyrene as an 'indicator' of initiating polynuclear aromatic hydrocarbons, and phenol as an `indicator' of tumor-promoting phenolic components.

FALK *et al*. (322) reviewed the presence of B[*a*]P and other PAHs in numerous items to which humans are exposed, e.g., soot, carbon black, coal tar, pitch, paraffin, mineral oil, and tobacco smoke. Extrapolating from the numerous reports they reviewed, they stated:

> . . . certain polycyclic aromatic hydrocarbons are indeed carcinogenic for man and the most ubiquitous and potent of these is in all probability 3,4-benzpyrene . . .

FALK *et al*. stressed the importance of three publications concerning the effect of B[*a*]P on man, publications that stressed the similarity between the cellular changes observed in laboratory animals skin painted with B[*a*]P solutions and those observed in humans administered B[*a*]P to the skin inadvertently [CLAR (323)] or by design [COTTINI and MAZZONE (324)].

Other publications in 1964 include that of GALUSKINOVA (325) who reported the B[*a*]P content of ETS. Publications by HUBERT-HABART *et al*. (326), KRÖLLER (23), MÜLLER *et al*. (327), PERAKIS (328), PIETZSCH (21) plus the lengthy review article by WYNDER and HOFFMANN (77) dealt with some aspects of B[*a*]P in cigarette MSS. RODGMAN (65) summarized his eight-year research on the composition of cigarette MSS and the control of specific classes of compounds (PAHs, phenols, carbonyl compounds) in it.

1965 WYNDER and HOFFMANN (329) wrote (p. 93) that the use of 'chemical indicators' was suggested as a basis for prediction of the specific tumorigenicity of a given CSC. Their 'indicators' for cigarette MSS were described as follows:

> These include total smoke condensate as a base line for dose response, nicotine as an indicator of toxicity, benzo[*a*]pyrene as indicator for tumor-initiating polycyclic aromatic hydrocarbons, phenol as indicator for tumor-promoting acidic components, acrolein as indicator for ciliatoxic aldehydes, formic acid for ciliatoxic acids, and carbon monoxide as indicator for 'completeness' of combustion.

FREDRICKSON (330) proposed the use of expanded tobacco as a means to control MSS TPM yield and composition. Research on the proposal eventually led to several patents, e.g., (331). The use of expanded tobacco in the cigarette blend decreased the per cigarette yield of "tar", nicotine, and many other MSS components, including that of B[*a*]P and other PAHs (332).

RODGMAN and COOK (82) began a series of presentations on the results of their research from 1955 to 1965 on the composition of cigarette MSS. Examination of the text and slides of the presentations reveals that most of the details on the so-called MSS toxicants (PAHs, phenols, aldehydes and ketones) were included.

Other 1965 publications on PAHs and/or B[*a*]P in cigarette MSS included the following: determination of B[*a*]P and other PAHs in MSS by AYRES and THORNTON (87), generation of PAHs by pyrolysis of tobacco components by BADGER *et al*. (137), analysis of PAHs in MSS by CARUGNO and ROSSI (90) and GUVERNATOR *et al*. (88), the effect of filter-tip materials on B[*a*]P in MSS by DIKUN *et al*. (333), the pyrogenesis of PAHs from tobacco additives by KRÖLLER (24, 334), the dependence of cigarette MSS on tobacco type by LIPP (335), estimation of B[*a*]P in MSS by PAILER *et al*. (198), and the effect of treatment of cigarette paper on MSS PAHs by PYRIKI *et al*. (336).

1966 LAZAR *et al*. (310), in a thorough study of B[*a*]P, reported that increasing the B[*a*]P content of CSC by as much as 30-fold gave no increase of carcinogenic activity on the skin of susceptible mice. They concluded that the "benzo[*a*]pyrene content is not important in the carcinogenicity of cigarette smoke condensate". Their finding on B[*a*]P in CSC agreed with the 1962 finding of ROE (309), but both the ROE and LAZAR *et al*. findings were at odds with the findings on doubling and tripling the level of 17 known tumorigenic PAHs in CSC subsequently described by HOFFMANN and WYNDER in 1968 (138).

WYNDER and HOFFMANN (337) again described their 1963 findings with nitrate-enriched (8%) tobacco on reduction of both the tumorigenicity of the CSC to mouse skin and the levels of tumorigenic PAHs, including B[*a*]P, in the CSC.

The results of research on PAHs in cigarette smoke with emphasis on B[*a*]P were reported in 1966 by GRIMMER *et al*. (338) (who also discussed B[*e*]P), KIRYU and KURATSUNE (89), KRÖLLER (339), RATHKAMP *et al*. (340), and VAN DUUREN *et al*. (25).

1966/1967 COOK and RODGMAN (341) examined the effect of various tobacco additives (tetravalent metal salts, cyclodextrins, citric acid) and several filter-tip materials on the per cigarette yield of PAHs. None was found to be effective.

1967 HOFFMANN and WYNDER (342) reported that addition of 8.3% sodium nitrate to their control cigarette tobacco resulted in a CSC with "a statistically significant reduction of the tumorigenicity" vs. the control CSC. In addition, they reported a 65% reduction in the per cigarette MSS yield of B[*a*]P and a 43% reduction of B[*a*]P on a per gram of CSC basis. *NOTE***:** At this point in time, HOFFMANN and WYNDER failed to detect NNAs in CSC from either the standard or the nitrate-enriched tobacco.

WYNDER and HOFFMANN (135) reported that the B[*a*]P content per milligram of "tar" had decreased over the years (from 1954) as Tobacco Industry cigarette designers incorporated various technologies, e.g., reconstituted tobacco (stem) sheet, (RTS) in the cigarette design.

NOTE: This trend continued thereafter with the increased usage of RTS, more efficient filters, the introduction and use of porous paper, filter-tip and paper additives, the introduction of filter-tip perforations for increased ventilation, and the use of expanded tobacco [see the 1979 Surgeon General's report (111) discussed below].

WYNDER and HOFFMANN described in more detail their use of B[*a*]P and phenol as 'indicators' [see p. 517 in (135)]:

> BaP and phenol have been chosen as 'chemical indicators' of the tumorigenic activity of smoke condensates. They indicate the concentration of carcinogenic PAH by the value for BaP and that of phenol as a member of one group of tumor promoters present in tobacco 'tar' . . .

They also wrote [see pp. 625–626 in (135)]:

> Without belaboring the point as to whether BaP as such contributes to the carcinogenicity of tobacco smoke condensate, we can certainly agree that the concentration of BaP may be regarded as an 'indicator' of carcinogenic PAH in tobacco smoke condensate . . . While BaP and other carcinogenic PAH can by themselves account for only a small portion of the total tumorigenic activity of cigarette smoke condensate, probably less than 2%, they are, nevertheless, of obligatory importance as tumor initiators . . .

They noted [see p. 626 in (135)]:

> . . . phenol and some of its derivatives have been shown to possess tumor-promoting activity . . . It should be noted, however, that a reduction of phenols in tobacco smoke condensate has not led to a concomitant reduction of tumorigenicity in the corresponding 'tar' . . .

PAPPAS and BINOPOULOS (92) identified B[*a*]P and B[*a*]A in CSC by thin-layer chromatography. They also identified several alkyl-PAHs, e.g., alkyl derivatives of anthracene, phenanthrene, pyrene, fluoranthene, chrysene, and B[*a*]A.

1968 WYNDER and HOFFMANN (343) reiterated their use of B[*a*]P and phenol as 'indicators' of tumor initiators and tumor promoters, respectively.

HOFFMANN and WYNDER (138) summarized the results of their studies with nitrate-enhanced tobacco cigarettes: Significant reduction in deliveries of PAHs, including B[*a*]P, and significant reduction of tumorigenicity of CSC to mouse skin. They noted the dramatic increase in nitroalkanes, nitroalkenes, and nitrobenzene in the MSS from nitrate-enhanced tobacco and speculated on the possible increase in NNAs. They also attempted to counteract the reports by ROE (309) and LAZAR *et al.* (310) that substantial enhancement of the B[*a*]P content of CSC did not increase the tumorigenicity of the CSC to mouse skin by describing the increase in tumorigenicity of CSC whose content of 17 identified PAHs was doubled and tripled. The added 17 PAHs included several classified by WYNDER and HOFFMANN as tumorigenic, e.g., B[*a*]P, DB[*a,h*]A, B[*a*]A, benzo[*k*]fluoranthene, indeno[1,2,3-*cd*]pyrene. The only way the 17 PAHs became effective tumorigens was when they were co-administered with an initiator either 10 mg of 3-methyl-1*H*-indole (skatole) or 10 mg of the indole-carbazole fraction from CSC.

HOFFMANN and WYNDER (138) also noted:

> A second potential for reducing the carcinogenic hydrocarbons in tobacco smoke and the tumorigenicity of the resulting "tar" was demonstrated by the use of reconstituted tobacco sheets with and without the addition of nitrate.

In 1968, STEDMAN (109) wrote:

> . . . data have appeared which show that levels of benzo[*a*]pyrene are *not* directly related to the tumor-initiating properties [of cigarette smoke condensate [ROE (309), LAZAR *et al.* (310)]. On the other hand, reduction in benzo[*a*]pyrene has been employed as one of several criteria to determine the relative tumorigenicity of different smoke samples [WYNDER and HOFFMANN (329)], and biological data confirming the use of such criteria have been obtained [HOFFMANN and WYNDER (135), WYNDER (83)]. Thus, the question of what chemicals compounds are responsible for the tumorigenic activity of smoke cannot be answered categorically.

CHAMBERLAIN *et al.* (344) and MARTIN and DOBBINS (345) described analyses for the PAH-rich fraction from cigarette MSS.

1968/1980 Data from the NCI "less hazardous cigarette" program [GORI (93, 94), NCI (139)] indicated that B[*a*]P was not an 'indicator' in cigarette MSS for either the specific tumorigenicity of the CSC or the per cigarette yields of tumorigenic PAHs or PAHs with four or more fused rings. In the study of the first two sets of cigarettes (93), the per cigarette MSS yields of B[*a*]A, B[*a*]P, and phenanthrene were quantitated. In the study of the last two sets of cigarettes, the phenanthrene was not quantitated (94).

1969 RATHKAMP and HOFFMANN (346) demonstrated that the MSS from nitrate-enhanced tobacco cigarettes showed reduced levels of tumorigenic PAHs, reduced tumorigenicity to mouse skin, and increased levels of nitrobenzene and nitroalkanes. Later studies revealed that increased nitrate on tobacco yields increased levels of NNAs in the MSS.

SELIKOFF *et al.* (347) conducted a study of male members of the roofers' union and their exposure to high levels of B[*a*]P. They estimated that daily a nonsmoking roofer might inhale the B[*a*]P in the MSS of 715 cigarettes. Even if all the men studied had been nonsmokers (which they were not) and worked at roofing only one day per week:

> . . . they still would have inhaled at least as much benzo[*a*]pyrene per year as very heavy cigarette smokers in the general population. If this hypothesis were true their death rate should have been at least two or three times as high as the lung cancer death rate of all men in the U.S. We conclude that if a high level of exposure to benzo[*a*]pyrene has any relation to lung cancer, the effect must be small . . . if a high level of occupational exposure to benzo[*a*]pyrene by way of inhalation results in little if in any increase in the risk of lung cancer — then it seems unlikely that the extremely small amount of benzo[*a*]pyrene in cigarette smoke can account for the high degree of association between cigarette smoking and lung cancer.

As a result of their study on the tumor-promoting activity of components in CSC, WYNDER and HOFFMANN (348) concluded that the carcinogenic PAHs are concentrated in a subfraction of the neutral portion of tobacco "tar" and their tumor initiating activity was found to be about 20 times greater than could be explained by the B[*a*]P content. They assumed other tumor initiators of PAH or similar structural types were

present in the subfraction. In support of these statements, WYNDER and HOFFMANN cited their own publications (135, 343).

In response to the 1969 study by SELIKOFF *et al.* (347), a news release of the AMERICAN CANCER SOCIETY (349) stated:

> . . . a known cancer causing agent in experimental animals may have to be ruled out as a cause of lung cancer in man.

1969 WYNDER and HOFFMANN (350) tabulated their recommended 'indicators', the same ones listed previously in WYNDER and HOFFMANN (329).

COMMINS (351) studied the formation of PAHs during the pyrolysis of hydrocarbons and LEDFORD *et al.* (352) described the separation of PAHs by high resolution gas chromatography. Both reports emphasized B[*a*]P.

1970 DONTENWILL *et al.* (353) reported that the frequency of metastases was lower in mice treated with CSC than in those treated with B[*a*]P.

HEGE (354) determined the per cigarette MSS B[*a*]P yields of 28 top-selling U.S. cigarette brands. Some years earlier, in 1962, CARPENTER (355) had determined the MSS B[*a*]P yield for three U.S. cigarette brands. The introduction of various cigarette design technologies between 1962 and 1970 resulted in a substantial decrease in the B[*a*]P yields of the brands common to both analyses.

WYNDER and HOFFMANN (356) wrote:

> The best known carcinogen [in CSC] is benzo(a)pyrene (BaP). By itself, this compound cannot account for the total carcinogenic activity of the whole "tar", nor for the initiating action of the "tar"; yet it represents a good indicator of the carcinogenic activity of different tobacco "tars' . . .

With the identification of several volatile and nonvolatile NNAs in MSS and the eventual identification of tobacco-specific *N*-nitrosamines (TSNAs) in tobacco and tobacco smoke, the number of published reports on the various NNAs increased while the number of reports on PAHs decreased. For many of the smoke components of concern there were alternate sources of exposure. The one exception, obviously, is the TSNAs.

1971 VAN DUUREN *et al.* (357) tested numerous tobacco leaf and tobacco smoke components for cocarcinogenic activity on mouse skin by simultaneous and repeated application with B[*a*]P. Various tumor-promoting substances were also tested, and they showed varied results, ranging from significant cocarcinogenic activity to weak activity to no activity. These results indicated the complexity of the problems encountered in experimental tobacco carcinogenesis.

KRASNYANSKAYA (358) examined the effects on the respiratory tract in 95 rabbits of chronic exposure to cigarette MSS. One group was pretreated intratracheally with B[*a*]P. Though premalignant changes were found in the B[*a*]P-treated animals, no lung malignancies were observed after a 4-year exposure.

CHAKRABORTY *et al.* (359) described the per cigarette reduction of a number of PAHs, including B[*a*]P, in the MSS of cigarettes where the tobacco was treated with bismuth oxide or calcium oxalate.

1972 SYDNOR *et al.* (360) reported the results of tumor-induction experiments with an aqueous extract of CSC prepared from a commercial cigarette brand together with a similar B[*a*]P solution.

The following summarizes the findings in two other 1972 investigations: In a study with 16,17-^{14}C-radiolabeled *n*-dotriacontane added to a cigarette tobacco blend, JENKINS *et al.* (361) reported that the labeled compound contributed only 1/1500 of the B[*a*]P in MSS, and 1/4200 of the B[*a*]P in SSS. LEFFINGWELL and WORRELL (362) investigated the effect of aliphatic hydrocarbon-treated filter tips on the B[*a*]P in MSS. The various aliphatic hydrocarbons studied had little effect on the B[*a*]P yield in the MSS.

1973 In contrast to the findings of other investigators who reported phenol to be a promoter for tumorigenic PAHs such as B[*a*]P, VAN DUUREN *et al.* (363) reported that the tumorigenicity of B[*a*]P was inhibited, at least partially, by phenol.

CHORTYK and SCHLOTZHAUER (364) completed an excellent review of the pyrogenesis of many components appearing in tobacco smoke that were not tobacco components. The pyrogenesis of PAHs was discussed in detail.

1973/1975 KLIMISCH (365) and HAEBERER *et al.* (366) reported on methods to analyze PAHs in mainstream CSC.

1974 In a Tobacco Chemists' Research Conference (TCRC) presentation, HECHT *et al.* (367) noted:

> In previous years, we have reported on a number of tumor initiators in tobacco smoke, especially polynuclear aromatic hydrocarbons . . . These components alone, in their known concentrations, cannot explain the biological activity of cigarette smoke condensate when tested on experimental animals.

1974/1979 Between 1974 and 1979, the investigators at the USDA in Athens, GA conducted a massive study on the PAHs in CSC. The overall result was the complete or partial identification of over 500 PAHs. The identifications classified as partial involved those alkylated PAHs where the position of the alkyl substituents was not precisely defined. Journal publications and/or conference presentations involved the following: SNOOK *et al.* (16, 17, 18, 19, 368, 369, 370), SEVERSON *et al.* (371, 372, 373, 374, 375, 376), and AKIN *et al.* (377). Several major points of interest evolved from this research: 1) No PAH was identified that could be classified as a "supercarcinogen", 2) many mono-, di-, tri-, tetra-alkyl-PAHs were identified despite assertions by some that their pyrogenesis during the smoking process was highly improbable, 3) several PAHs isomeric with 1,2-dihydrobenz[*j*]aceanthrylene (cholanthrene) were reported but 1,2-dihydrobenz[*j*]aceanthrylene was not identified.

1975 HAEBERER and CHORTYK (378) reviewed the advances in high pressure liquid chromatography (HPLC) in the separation of PAHs in CSC. At that time, complete separation of B[*a*]P and B[*e*]P could not be accomplished by HPLC.

1975/1976 MCCANN *et al.* (379) and MCCANN and AMES (380) bioassayed 300 tumorigenic compounds, including B[*a*]P, in the Ames *Salmonella typhimurium* test: B[*a*]P was mutagenic.

1976/1978 VAN DUUREN *et al.* (381) reported that several weakly carcinogenic or noncarcinogenic PAHs, inclu-

ding pyrene[a], fluoranthene[a], and B[*e*]P[a], are cocarcinogenic when repeatedly applied with B[*a*]P to mouse skin.

[a] PAH identified in tobacco smoke.

1977 TSO and CHAPLIN (98) described the correlation between various tobacco components and various MSS components for a variety of flue-cured tobaccos. Two PAHs, B[*a*]P and B[*a*]A, were determined in the MSS from the different tobacco varieties. Correlations were presented between the level of them in MSS and the response in several short-term bioassays. No mouse skin-painting studies were involved. Several years later, TSO *et al.* (382) reported similarly on tobacco and smoke components for burley tobaccos. Only B[*a*]P was determined in the MSSs. The conclusion for burley was that the CSC B[*a*]P content showed no significant differences among the various burley tobaccos or stalk positions on the plant.

1977 THE ROYAL COLLEGE OF PHYSICIANS (173) characterized PAHs as one of the two compound classes which are the chief tumor initiators in tobacco smoke. The other tumor-initiating class of compounds in tobacco smoke is the NNAs.

MARRIOTT and WEAVING (179) described a procedure for determining the per cigarette yield of B[*a*]P in the condensates from tobacco and tobacco substitute smoking materials.

1978 HOFFMANN *et al.* (383) discussed cigarette design technology (filtration, American-type tobacco blend, reconstituted tobacco sheet, expanded tobacco, etc.) that, between 1955 and 1978, reduced B[*a*]P levels both per cigarette and per milligram of "tar." They also noted that their studies showed B[*a*]A significantly inhibited the mouse-skin tumorigenicity of B[*a*]P.

1979 The U.S. SURGEON GENERAL (111) reported:

> . . . levels of carcinogenic polynuclear aromatic hydrocarbons in tobacco smoke are well below their practical threshold as complete mouse skin carcinogens, but their role in tobacco smoke condensate is definitely that of a tumor initiator. Benzo[*a*]pyrene was categorized in this report as a complete carcinogen . . . Also, benzo[*a*]pyrene is often used generally as an indicator of PAH levels and specifically as an indicator of the carcinogenic potential of the smoke as measured in animal experiments. However, this 'indicator' concept can be applied only to smoke deriving from cigarettes primarily made up of the same precursor material, i.e., tobacco leaves.

NOTE: The Surgeon General's 1979 comments on B[*a*]P should be compared (or contrasted) with his 1981 statements (112).

1979/1980 In three separate studies, MCMAHON *et al.* (384), RINKUS *et al.* (142), and BARTSCH *et al.* (143) found that several tobacco smoke components, including B[*a*]P, were mutagenic in the Ames test with *Salmonella typhimurium*.

1980 In a discussion of the extensive research conducted since the 1950s to define the key tumorigenic factor in cigarette smoke, COULTSON stated (385):

> Whether it's benzo[a]pyrene or not, nobody really knows. More work has been done on benzo[a]pyrene to prove it to be the causative agent in cigarette smoking than I think on any other chemical for any disease that I know. And yet the point is, you can't prove it.

1981 In contrast to statements in his 1979 report (111) (see above), the U.S. SURGEON GENERAL (112) wrote in his 1981 report:

> The contribution of B*a*P or PAH in general to mouse skin carcinogenesis by cigarette smoke condensate cannot be fully measured at this time. WYNDER and HOFFMANN (135) found a correlation between B*a*P levels and carcinogenic activity of smoke condensates from several types of cigarettes. A much larger series of experimental cigarettes was studied in the smoking and health program of the National Cancer Institute. No significant dependence of carcinogenic potency on B*a*P content was observed [93, 94, 139].

With regard to the use of B[*a*]P as an 'indicator' for other tumorigenic PAHs with four or more rings, the SURGEON GENERAL noted in 1981 (112):

> Among smoke condensates from 98 experimental [and some 40 control and reference] cigarettes, the correlation coefficient between [benzo[*a*]pyrene] and benz[*a*]anthracene content was 0.78 [BAYNE 386]. Although highly significant, the value is sufficiently low to indicate that real differences do exist in the ratios of these cyclic molecules in the various cigarette smokes.

1982 From a detailed literature review, KLUS and KUHN (99) described the relationship between contents in cigarette MSS and SSS of a variety of smoke components. For PAHs classified as tumorigenic, the SSS:MSS ratio for B[*a*]A and B[*a*]P were presented, 2.1 and 2.7, respectively. Other PAH ratios reported included those for pyrene (1.9), phenanthrene (2.1), naphthalene (16.5), 1-methylnaphthalene (26.1), and 2-methylnaphthalene (29.4).

The 1982 report of the U.S. SURGEON GENERAL classified B[*a*]P as a "toxic and tumorigenic agent" in cigarette smoke with amounts of 10 to 50 ng/cig (113). These MSS B[*a*]P yield data were those reported earlier by HOFFMANN *et al.* (383).

NOTE: A per cigarette yield for B[*a*]P in cigarette MSS in excess of 20 ng indicates that the data are probably from pre-1970 cigarettes. In this 1982 report, the Surgeon General also discussed briefly the relationships in experiments with laboratory animals among B[*a*]P, its various hydroxylated and epoxylated metabolic products, and tumorigenicity in various laboratory animal species and strains.

1983 DEMARINI (387), in his review of the mutagenicity of tobacco smoke and tobacco smoke condensate, noted the differences between the mutagenicity (as measured in the Ames test with *Salmonella typhimurium*) and tumorigenicity (as measured in mouse skin-painting studies). Previously, WYNDER and HOFFMANN (135) had claimed that the specific tumorigenicities of CSCs from different tobacco types, their B[*a*]P contents, and their phenol contents followed the following sequence for the different tobacco types:

flue-cured > Oriental > Maryland > burley

As noted by DEMARINI (and others) for specific mutagenicities of cigarette MSS total particulate matter, the following contrasting sequence was found:

flue-cured < Oriental < Maryland < burley

1985 YAMAMOTO *et al.* (388) described the effect of cigarette circumference on the yield and composition of MSS. Included were data on the per cigarette yield of B[*a*]P.

1985/1986 IARC, in its deliberations and subsequently published monograph on tobacco smoking (27), noted that from its review of the PAHs in tobacco smoke and their potential carcinogenic activity, there was sufficient evidence for B[*a*]P to be considered carcinogenic to laboratory animals. No specific comment was included on the carcinogenicity of B[*a*]P to humans. The IARC briefly commented on the difference in per cigarette MSS yield of B[*a*]P for different types of tobacco. The following summarizes the studies discussed:

	Benzo[*a*]pyrene, ng/cig		
Tobacco type	Wynder and Hoffmann (134)	Lipp (335) [a]	Robb *et al.* (178)
Flue-cured	53	28–37 [b]	26
Oriental	44	23–30 [b]	—
Burley	24	16–22 [c]	11
Maryland	18	—	—

[a] The B[a]P analyses were provided by Grimmer.
[b] The analysis involved four different samples of the tobacco type specified.
[c] The analysis involved three different burley tobacco samples.

1987 The Registry of Toxic Effects of Chemical Substances (RTECS) (389) reviewed a variety of studies on the administration of PAHs to laboratory animals by a variety of routes. The RTECS rated as “equivocal” the results, defined as "uncertain but seemingly positive", from inhalation studies with massive doses of PAHs, including B[*a*]P, which resulted in “lung neoplasms”. The RTECS findings were cited by AVIADO (390) in his response to the EPA after the issuance of its 1990 draft document in which it recommended that ETS be classified as a Group A (human) carcinogen.

1988 MAGA (50) summarized the human dietary exposure to B[*a*]P and cited the estimate of VAESSEN *et al.* (51) that the daily B[*a*]P exposure of Dutch citizens averaged about 0.5 µg/day (500 ng/day). This intake via ingestion is about the same as the 1991 estimate by WALDMAN *et al.* (52) (up to 500 ng/day, see below) but much less than that of HATTEMER-FREY *et al.* (53) (2200 ng/day, see below).

1990 HOFFMANN and HECHT (115) used the 1986 IARC data (27) and listed B[*a*]P as one of 43 “significant tumorigens” in tobacco and tobacco smoke. This list was subsequently used by the EPA (145) in its attempt to have ETS classified as a Group A (human) carcinogen. HOFFMANN and HECHT noted:

> The tumorigenicity[a] of inhaled [benzo[*a*]pyrene] has also been established in Syrian golden hamsters . . . These findings, taken together with the results of bioassays on mouse skin, provide strong evidence for the role of PAH as tumor initiators in tobacco-related respiratory carcinogenesis . . . The levels of exposure to PAH as experienced by smokers are not inconsistent with their potential role as causative agents for respiratory tract cancer . . .
>
> [a] No squamous cell carcinoma was produced in the animals exposed to levels of B[*a*]P far in excess of that encountered in cigarette smoke inhalation.

The authors then attempted – in an unusual comparison – to correlate the results of mouse skin-painting with a solution of B[*a*]P and the intratracheal administration of B[*a*]P on ferric oxide to the induction of respiratory tract cancer in cigarette smokers. Caution against attempts to use this type of extrapolation – different mammalian species and different modes of administration of the agent in question – had been described in 1941 by SHEAR and LEITER (391) in their description of the factors that influence the specific tumorigenicity of a specific compound or element.

HOFFMANN and HECHT also wrote:

> It has been clearly demonstrated that the most tumorigenic fractions . . . are those with highly concentrated PAH . . . However, PAH by themselves do not account for the tumorigenic activity on mouse skin induced by the total particulate matter.

With regard to 4-(methylnitrosamino)-1-(3-pyridinyl)-1-butanone (NNK), HOFFMANN and HECHT commented that NNK has not been tested for tumorigenicity by inhalation.

In conjunction with ETS, EATOUGH *et al.* (392) discussed the concentration of components in cigarettes SSS and the SSS:MSS ratio of each. Of the dozen or so MSS PAHs listed as tumorigenic by IARC (27), HOFFMANN and WYNDER (114), and HOFFMANN and HECHT (115), EATOUGH *et al*. presented MSS and SSS data for only B[*a*]P and indeno[1,2,3-*cd*]pyrene.

1991 HATTEMER-FREY and TRAVIS (53) estimated the human exposure to B[*a*]P from inhalation and ingestion. They concluded:

> . . . the food chain is the dominant pathway of human exposure, accounting for about 97% of the total daily intake of B*a*P. Inhalation and consumption of contaminated water are only minor pathways of human exposure. The long-term average daily intake of B*a*P by the general population of the U.S. is estimated to be 2.2 µg per day. Cigarette smoking and indoor activities do not substantially increase human exposure to B*a*P relative to exposures to background levels of B*a*P present in the environment . . . we conclude that ingestion of food items contaminated with B*a*P may pose a serious health threat to the U.S. population.

In their study, WALDMAN *et al*. (52) directly monitored human B[*a*]P exposures via inhalation and ingestion, during three separate 14-day periods. They summarized their findings:

> Among the study subjects, the range and magnitude of dietary exposure (2 to 500 ng/d) were much greater than inhalation (10 to 50 ng/d).

It should be noted that this was their summary despite “the systematic omission of liquid foods and beverages”. This omission is curious since it has been known for many years that coffee, tea, milk, and other beverages are dietary sources of B[*a*]P [WIDMARK (393), FIESER (225), KURATSUNE and HUEPER (247), DANSI and ZANNINI (394), MAGA (50)].

It is interesting to note that none of the reports by MAGA (50), GRASSO (193), HATTEMER-FREY *et al.* (53), and WALDMAN *et al*. (52) mentions the complex formation between PAHs by purines to form water-soluble compounds. This property was first reported by WEIL-MALHERBE in 1946 (395). Thus, B[*a*]P from a food source exposed to the caffeine in a beverage (coffee, tea, cola) could be converted to a water-soluble compound. This water-solubility phenomenon was used by ROTHWELL and WHITEHEAD in their procedure to isolate the PAH fraction from cigarette MSS (396). Essentially, their procedure was the reverse of the usual

procedure where the PAH fraction was concentrated in hexane by partitioning the CSC between hexane and aqueous ethanol.

1992 GUERIN *et al*. (103) in their book on ETS described the levels of B[*a*]P and B[*a*]A in various atmospheres with and without tobacco smoke present. In many cases, the B[*a*]P contributed from ETS is minor compared to the contribution from other sources.

1993/2001 In a series of publications on the cigarette and its yield of toxic MSS components, HOFFMANN and his colleagues listed B[*a*]P as a significant tumorigen (116, 117, 118, 119, 120, 397). HOFFMANN *et al*. [see Figure 4 in (397)] presented a chart to show the decrease in the B[*a*]P yield from 1959 through the mid-1990s but the first part of it, from the early 1950s to 1975, differs somewhat from the B[*a*]P chart presented by WEBER (398) in 1976 and included in the 1979 report on smoking and health by the SURGEON GENERAL (111).

1994 OSHA (147) prepared its own list of 43 significant tumorigenic components in tobacco smoke. Its list differed from that of HOFFMANN and HECHT (115). The OSHA list contained only 42 components. It appeared that ^{210}Po may have been omitted. B[*a*]P was among the twelve PAHs listed.

1996 From the results of their experiments on the effect of the B[*a*]P metabolite BPDE (7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene) on HeLa cells, DENISSENKO *et al.* (399) claimed that their results provided proof that the B[*a*]P in cigarette smoke was involved in lung cancer initiation. Of the more than a dozen known B[*a*]P metabolites, DENISSENKO *et al.* selected for their study the one demonstrated to be the most tumorigenic. The following should be considered: The molecular weight of B[*a*]P is 252; the molecular weight of the specified B[*a*]P metabolite BPDE is 300. In this DENISSENKO *et al.* study, cell system samples (weight unspecified) were treated with three BPDE dose levels, 1 μM, 2 μM, and 4 μM [see Reference 9 in (399)]. These dose levels represent 300 μg, 600 μg, or 1200 μg of BPDE, respectively; i.e., 300000 ng or 600000 ng or 1200000 ng of BPDE, respectively. If the metabolism of B[*a*]P yielded 100% of the specific BPDE, then the dose levels represented the BPDE derived from 252000 ng or 504000 ng or 1080000 ng of B[*a*]P, respectively. At a 20-ng/cig delivery, the cell system was treated with the BPDE from the B[*a*]P in the MSS from 12600 or 25200 or 50400 cigarettes, respectively.

In their review of contributions to analytical technology described at the first fifty TCRCs, GREEN and RODGMAN (400) summarized the twenty-seven presentations on B[*a*]P. A B[*a*]P study was first described at the 9th TCRC in 1955 by ALVORD and CARDON (236).

1999 In his list of eight tumorigenic PAHs in cigarette MSS, HECHT (149) listed DB[*a,h*]A at a yield of 4 ng/cig. HECHT rated DB[*a,h*]A as a "substantially stronger lung tumorigen" than B[*a*]P but noted that its yield (4 ng/cig) was much lower in cigarette MSS than that of B[*a*]P (2040 ng/cig), a range that included data from cigarette MSS analyzed three to four decades earlier.

2000 Categorizing them as supported or not supported, RODGMAN *et al*. (150) listed many of the assertions made over the years about PAHs in cigarette MSS. Chief among them were the following: 1) The difference of opinion on the major precursors in tobacco of PAHs in smoke, 2) the difference of opinion on the mechanism of their formation, 3) the view that alkylated PAHs could not be formed from tobacco components during the smoking process, 4) the presence in mainstream CSC of a "supercarcinogenic" PAH, 5) B[*a*]P as the 'indicator' of the specific tumorigenicity of CSC, and 6) the fate of a tobacco component pyrolyzed individually under conditions similar to the burning zone of a cigarette was equivalent to the fate of that component when pyrolyzed during the tobacco smoking process.

In their review of the "IARC Group 2A Carcinogens" in cigarette MSS, SMITH *et al*. (151) briefly summarized the sources of exposure to B[*a*]P and noted that some exposure sources were much greater than cigarette smoke. Like DB[*a,h*]A, B[*a*]P is among the five PAHs found in heated foodstuffs (192, 193).

2001 Similar to the lists from IARC (27), HOFFMANN and his colleagues (114, 115, 116, 117, 118, 119, 120) and OSHA (147), FOWLES and BATES (121) listed 11 MSS PAHs as tumorigenic. They omitted chrysene because IARC no longer considered it a tumorigen. They listed B[*a*]A at 45 ng/cig and B[*a*]P at 9.9 ng/cig.

RODGMAN (8) noted that in the various cigarette design technologies developed and used to control not only "tar" and nicotine delivery but also the composition of MSS, one of the smoke components included in almost every analysis was the determination of the per cigarette MSS yield of B[*a*]P. To conform to the definition of a "less hazardous" cigarette, the decrease in MSS yield of B[*a*]P must be greater than the decrease in MSS "tar". This is accomplished by many of the eight cigarette design technologies considered significant in the design of a "less hazardous" cigarette.

2002/2003 When RODGMAN and GREEN (55) averaged the B[*a*]P data from several laboratories on the 1R4F Reference cigarette, a yield of 5.2 ng/cig was obtained. The MSS yields from the 1R4F cigarette are more representative of the modern cigarette than are the yields determined for commercial cigarettes in the late 1950s, the 1960s, and the 1970s. The early yield data were always included in the ranges cited in most of the toxicant lists compiled between 1986 and 2001

2003 RODGMAN (43) discussed the deficiencies in many of the lists published to describe the components of tobacco smoke, particularly cigarette MSS, that the authors of the lists classify as detrimental to the smoker's health.

2004 CORESTA issued its description of a standard method for the quantitative determination of B[*a*]P in CSC (401).

2005 KALAITZOGLOU and SAMARA (154) described the distribution of PAHs between the particulate and vapor phases of the MSSs from a variety of cigarette types. B[*a*]P was quantified in the particulate phase of each cigarette type but was not detected in the vapor phase.

6 SOME ADDITIONAL COMMENTS

It is interesting to note that for several decades investigators have commented on MSS PAHs as follows: WYNDER and WRIGHT (231) and WRIGHT and WYNDER (232) in 1955

stated:

> . . . the concentration in which B[*a*]P seems to be in cigarette tar is insufficient to account for the observed carcinogenic activity to mouse epidermis.

And again in 1957 (237):

> . . . the benzpyrene content of the total tar as well as the active fractions is far too low to account alone for the positive results [in laboratory animal]. So far, no carcinogens have been identified in large enough quantity in tobacco tar or its fractions to account for the observed activity.

WYNDER and HOFFMANN in 1959 commented (15):

> From the amount in which these materials [PAHs, including B[*a*]P] have been found in tobacco smoke condensate it was evident that these, by themselves, could not account for the total biological activity observed.

And reiterated their statement in 1961 (297):

> The polynuclear aromatic hydrocarbons are mainly formed during the combustion of tobacco. The tobacco of our standard cigarettes contains only very minute quantities of benzo[*a*]pyrene (0.02 ppm). A bioassay indicates that these polycyclic hydrocarbons of the condensate by themselves, however, can account for not more than 3 per cent of the total biological activity.

Such comments were not limited to HOFFMANN and his colleagues, e.g., VAN DUUREN in 1958 (258) reported:

> . . . the carcinogenic hydrocarbons found so far do not by themselves appear to be present in sufficient concentrations to account for the observed activity.

In 1963, WYNDER and HOFFMANN (134) stated:

> Biostatistical calculation showed that only about three percent of the tumorigenicity of cigarette smoke condensate can be explained by the presence of the polycyclic hydrocarbons.

In its 1964 report on smoking to the U.S. Surgeon General, the ADVISORY COMMITTEE (127) wrote in Chapter 6:

> . . . the benzo[*a*]pyrene concentration of various tobacco and smoke preparations is only sufficient to account for a very small part of the carcinogenicity of these materials.

As a result of their study on the tumor-promoting activity of components in CSC, WYNDER and HOFFMANN (350) in 1969 concluded that the carcinogenic PAHs are concentrated in a subfraction of the neutral portion of tobacco "tar" and their tumor initiating activity was found to be about 20 times greater than could be explained by the B[*a*]P content. A year later, they reported (356):

> The best known carcinogen [in CSC] is benzo(*a*)pyrene (BaP). By itself, this compound cannot account for the total carcinogenic activity of the whole 'tar', nor for the initiating action of the 'tar'; yet it represents a good indicator of the carcinogenic activity of different tobacco 'tars' . . .

In 1974, HECHT *et al*. (367)

> In previous years, we have reported on a number of tumor initiators in tobacco smoke, especially polynuclear aromatic hydrocarbons . . . These components alone, in their known concentrations, cannot explain the biological activity of cigarette smoke condensate when tested on experimental animals.

Even more recently, in 1990 HOFFMANN and HECHT (115) stated with regard to PAHs and the TSNA NNK:

> It has been clearly demonstrated that the most tumorigenic fractions . . . are those with highly concentrated PAH . . . However, PAH by themselves do not account for the tumorigenic activity on mouse skin induced by the total particulate matter . . . It [NNK] has not been tested by inhalation.

Despite the multitude of statements pertinent to the PAHs in cigarette MSS and their contribution to the observed biological activity of CSC in laboratory animals, HECHT and HOFFMANN in 1991 appeared to have ignored these past assertions when, citing the above article (115), they wrote (402):

> We concluded that polynuclear hydrocarbons and NNK are the major carcinogens involved in lung cancer induction by cigarette smoke . . .

As described in Sections 2 through 5, extensive research has been conducted on the four MSS components during the past half century. Paralleling that research were numerous bioassays in which B[*a*]A, DMB[*a*]A, DB[*a,h*]A, and/or B[*a*]P were the compounds studied. Included in those bioassays were numerous ones on the inhibition of the tumorigenicity of the three powerful tumorigens DMB[*a*]A, DB[*a,h*]A, and/or B[*a*]P. Among the many compounds investigated in this regard were several components of tobacco smoke, most of which occurred in CSC at a much higher level than the three PAHs.

Table 5 summarizes many such studies conducted since the mid-1950s. Since very few, if any, inhibition studies have been conducted with B[*a*]A, it is not included in Table 5 as an inhibited tumorigen but it is included because of its inhibition of the tumorigenicity of DB[*a,h*]A and B[*a*]P when co-administered with them (128, 133). If B[*a*]A is a tumorigen, it is a borderline one (28). Additional references to antitumorigens or inhibitors may be found in the monograph by FAY *et al*. (403).

Most investigations of inhibition or antitumorigenesis involved the co-administration of similar doses of the tumorigen and the substance under study. It seems logical to assume that those showing significant inhibition of the tumorigenesis under those conditions would be even more effective if the ratio of inhibitor to tumorigen were much greater than 1:1, i.e., 5:1 or 10:1 or 20:1. Such is the case with many of the components in CSC that have been shown to inhibit the tumorigenicity of B[*a*]P or DB[*a,h*]A or DMB[*a*]A; their per cigarette MSS yields are much greater than those of the PAHs. Of course, the next question is: Will the inhibitors of the PAH tumorigenicity be equally as effective in a mixture as complex as CSC as when they are co-administered as a pair without any other substance, except the solvent, present?

Two experiments may have provided the answer. The first, described in detail in Section 5, was by WYNDER and HOFFMANN who demonstrated that the saturated aliphatic hydrocarbon fraction in CSC substantially reduced the tumorigenicity of B[*a*]P to mouse skin (165). With B[*a*]P and *n*-C_{31} and *n*-C_{35} hydrocarbons, two plentiful components of the hydrocarbon fraction, WYNDER and HOFFMANN reported that these hydrocarbons, at ratios of aliphatic hydrocarbon:B[*a*]P of 200:1 and 100:1, significantly inhibited the specific tumorigenicity of B[*a*]P at both ratios for both aliphatic Hydrocarbons. WYNDER and HOFFMANN also showed that increasing the level of the aliphatic hydrocarbon fraction in CSC substantially reduced the specific tumorigenicity of the CSC in mouse skin-painting experiments. The CSC specific tumorigenicity decreased from 40% to 24% by a 33% increase of the level of the saturated hydrocarbon fraction in CSC, i.e., increasing the fraction from 3% to 4% [see pp. 370–371 in (135)]. However, it should also be noted that HORTON *et al*. (404) reported in 1957 that an increase in the %TBA was observed by co-administration by skin painting of B[*a*]P and *n*-dodecane, the *n*-C_{12} aliphatic hydrocarbon in CSC. They reported a similar biological result with co-administration of *n*-dodecane and 1,2-dihydro-3-methylbenz[*j*]aceanthrylene.

Table 5. Mainstream smoke components reported as inhibitors or antitumorigens in mouse skin-painting studies

Mainstream smoke component	CAS No.	Effective against		
		DMB[*a*]A	DB[*a,h*]A	B[*a*]P
		Hydrocarbons, aliphatic		
Saturated aliphatic hydrocarbons [a]		—	—	Wynder and Hoffmann (165)
e.g., $C_{31}H_{64}$	630-04-6			
$C_{35}H_{72}$	630-07-9			
		Hydrocarbons, aromatic		
Benzene	71-43-2	—	Crabtree (217)	Crabtree (217)
Naphthalene	91-20-3	—	Crabtree (217)	Crabtree (217)
Anthracene	120-12-7	—	Crabtree (217)	Crabtree (217)
Phenanthrene	85-01-8	DiGiovanni *et al.* (218)	—	—
Fluoranthene	206-44-0	DiGiovanni *et al.* (218) ; Slaga *et al.* (405)	—	—
Pyrene	129-00-0	DiGiovanni *et al.* (218) ; Slaga *et al.* (405)	—	—
Benz[*a*]anthracene	56-55-3	—	Steiner and Falk (128)	Hoffmann and Wynder (133); Wynder and Hoffmann (311)
Benzo[*e*]pyrene	192-97-2	DiGiovanni *et al.* (218); Slaga *et al.* (405)	—	—
Benzo[*b*]triphenylene	215-58-7	Slaga and Boutwell (406); Slaga *et al.* (405)	Slaga and Boutwell (406); Slaga *et al.* (405)	—
		Alcohols		
α-4,8,13-Cyclodecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	57605-80-8	Saito *et al.* (407)	—	—
β-4,8,13-Cyclodecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	57605-81-9	Saito *et al.* (407)	—	—
		Acids		
1-Propene-1,2,3-tricarboxylic acid [c]	499-12-7	—	—	Kallistratos (408); Kallistratos and Fasske (409)
2-Propenoic acid, 3-(3,4-dihydroxyphenyl)- [d]	331-39-5	—	—	Wattenberg *et al.* (410)
2-Propenoic acid, 3-(3-hydroxy-4-methoxyphenyl)- [e]	537-73-5	—	—	Wattenberg (411)
2-Propenoic acid, 3-(2-hydroxyphenyl)-	614-60-8	—	—	Wattenberg *et al.* (410)
		Phenols		
Phenol	108-95-2	—	—	Van Duuren *et al.* (357)
Phenol, 4-methoxy-	150-76-5	—	—	Wattenberg *et al.* (410)
α-Tocopherol {vitamin E}	59-02-9	Shklar (412); Slaga and Bracken (413); Weerapradist and Shklar (414)	—	—
		N-Containing components		
Indole-3-acetonitrile	771-51-7	—	—	Wattenberg and Loub (415)
1*H*-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl- [f]	58-08-2	Perchellet and Boutwell (416)	—	—
Carbamic acid, ethyl ester	51-59-6	Shubik *et al.* (161)	—	—
		Miscellaneous components		
2*H*-Benzopyran-2-one [g]	91-64-5	Wattenberg *et al.* (417)	—	Wattenberg *et al.* (417)
3*H*-2-Furanone, dihydro-5-methyl- [h]	108-29-2	—	—	Wattenberg *et al.* (417)
Dioxin		Berry *et al.* (418); Cohen *et al.* (419); DiGiovanni *et al.* (420)	Berry *et al.* (418); Cohen *et al.* (419); DiGiovanni *et al.* (420)	Berry *et al.* (418); Cohen *et al.* (419); DiGiovanni *et al.* (420)
Maleic anhydride	108-31-6	Klein (421)	—	—
Selenium	7782-49-2	Shamberger (422)	—	—

Abbreviations: DMB[*a*]A = 7,12-dimethylbenz[*a*]anthracene; DB[*a,h*]A = dibenz[*a,h*]anthracene; B[*a*]P = benzo[*a*]pyrene.

[a] This fraction consists primarily of the *normal-, iso-* (2-methyl-), and *anteiso-* (3-methyl-) alkanes from C_{15} to C_{40}.

[b] Because of the various views on its tumorigenicity, ranging from inactive to very weak, studies – if any – on the inhibition of the tumorigenicity of benz[*a*]anthracene are not included in this Table.

[c] Aconitic acid. [d] Caffeic acid. [e] Ferulic acid. [f] Caffeine. [g] Coumarin. [h] α-Angelica lactone.

The second experiment involved the organic solvent extraction of tobacco to remove the phytosterols, terpenoids such as solanesol, and the saturated aliphatic hydrocarbons, the three component classes considered to be the major precursors in tobacco of PAHs in MSS (14, 364). The extracted tobacco, when smoked in cigarette form, yields 1) a CSC with a significantly reduced yield of PAHs (8) and 2) a CSC that shows reduced specific tumorigenicity on mouse skin painting. However, the PAH reduction was reported to be much greater than the tumorigenicity reduction. A possible reason is the following: The extraction not only removed the major PAH precursors but also removed much of the inhibitors such as the saturated hydrocarbons, α-tocopherol, and the cyclodecatriene-1,3-diols, Thus, the PAHs generated during smoking from nonextractable tobacco components such as cellulose, pectins, and starch are accompanied in the MSS by extremely low levels of the inhibitors.

While the number of reported studies on various aspects of B[*a*]P in cigarette MSS far exceed the number of studies devoted to the other three PAHs discussed herein, it is interesting to note that efforts to improve the analytical methodology to determine the per cigarette MSS yield of B[*a*]P have continued for over half a century. Almost every aspect of new and improved analytical methodology has been applied to the determination and quantitation of the B[*a*]P yield. Some have been used individually, others have been used sequentially in concert. Over the years, the various refinements have permitted the determination of B[*a*]P in the MSS from fewer and fewer cigarettes. Table 6 summarizes the chronology of many of the various studies and methodologies used to date. In some instances, all or part of the title of the article provides a clear indication of the analytical methodology described.

Is the zeal shown by numerous investigators over more than fifty years in developing improved analytical procedures for B[*a*]P in MSS due to the interpretation of the results of many hundreds of biological studies conducted with B[*a*]P on laboratory animals and extrapolation of such results to the human situation (31, 32, 423)? Are the 1941 cautionary comments by SHEAR and LEITER (391) on the dangers of extrapolation from laboratory animal data (skin painting with a concentrated solution) to the human situation (exposure by inhalation to an aerosol) being ignored? How many investigators consider the views expressed in 1987 by AMES *et al.* (424)? AMES *et al.* discussed the reasons why laboratory animal cancer tests cannot be used to predict absolute human risks and concluded:

> It is not scientifically credible to use the results from rodent tests done at the MTD (maximally tolerated dose) to directly estimate human risks at low doses.

More recently in the late 1990s, AMES and his colleagues have essentially reiterated the SHEAR and LEITER comments in their caution against such extrapolations as follows: 1) Extrapolation of cancer potency results from maximum tolerated dose studies to real-life exposures is not scientifically supportable. 2) Extrapolation of cancer potency results in rodents to humans cannot be validated (GOLD *et al.* 425, 426). However, despite these views, AMES *et al*. obviously did not dismiss the hazards incurred from tobacco or dietary saturated fat (424).

It is interesting to note the wealth of data on exposure to tumorigens from sources other than tobacco smoke. Noted investigators with hundreds of publications on their research results are given short shrift by government agencies, including the agency for which several of them worked. In the USA, the several hundred publications by HUEPER, a long-time member of the National Cancer Institute and its predecessor, on environmental causes of cancer, including lung cancer, are seldom discussed in detail by investigators, institutions, or government agencies strongly supportive of the lung cancer-smoking literature (427) [d]. E.g., in the ADVISORY COMMITTEE 1964 report (127), a lengthy HUEPER publication was dismissed in a sentence in which HUEPER was cited as being among "a number of investigators, though accepting the existence of an association [between lung cancer and smoking] have questioned its significance in terms of a causal hypothesis". A member of the US NCI (National Cancer Institute), SHEAR pioneered much research in the tumorigenicity of PAHs (428) and, as noted previously (391), provided the guidelines for discussions and interpretations of results of carcinogenesis studies with laboratory animals. Numerous PAHs have been identified and quantitated in a variety of foodstuffs and beverages (50, 51, 54, 193), but the presence of the tumorigenic ones does not receive the same extensive coverage as do the same tumorigenic PAHs in tobacco smoke.

In the U.K., KENNAWAY was a pioneer in the study of carcinogenesis long before the PAHs DB[*a,h*]A, B[*a*]P, or DMB[*a*]A were subsequently found in many of the tars or mixtures he investigated. KENNAWAY and his colleagues were the first to report the tumorigenicity to laboratory animals of the PAHs DB[*a,h*]A (29), B[*a*]P (30, 44), and DMB[*a*]A (157). Overall, they spent nearly four decades studying the effect of various exposures (foodstuff, air pollutants) on the incidence of cancer (70, 429).

In Germany, GRIMMER and his colleagues not only studied PAHs in tobacco smoke (95, 299, 338) but also studied their presence and levels in a variety of materials to which humans are exposed (430). While numerous PAHs, including B[*a*]A, DB[*a,h*]A, B[*a*]P, and DMB[*a*]A, were identified in many media in the various studies cited, none of the studies matched the detail to which the PAHs in tobacco smoke were subjected by the personnel at the US Department of Agriculture (16–19, 97, 265, 366, 368–375).

This annotated chronology of reports from the late nineteenth century to date on the chemical and biological properties of B[*a*]A, DMB[*a*]A, DB[*a,h*]A, and B[*a*]P provides an historical perspective of the studies conducted on these PAHs. Many of the studies provided landmark, clear, and irrefutable discoveries (29, 30, 44), some looked at these PAHs only tangentially, others provided more queries than answers to the biological importance of the PAHs in question. A few of the reports by noted scientists, educational institutes and government agencies lacked consistency in both the reporting of relevant data and in a few cases erred in promoting fear versus valid scientific facts. Yet, as time has passed and more credible information has become available, perhaps *the* truth can prevail. Now, after more than a century of chemical research and nearly 75 years of biological studies on these four PAHs, several conclusions can be drawn from the past

[d] The citations to the studies of HUEPER, E.L. KENNAWAY, and GRIMMER are presented in a somewhat unusual fashion to stress the exceptional extent of their studies over many years on environmental exposures to PAHs. Their studies are listed chronologically but not all are listed.

Table 6. Chronology of analytical methodology used in the determination of benzo[*a*]pyrene in cigarette mainstream smoke

Date	Titles	Methodologies [a]	Reference
1956/1958	The analysis of cigarette smoke condensate I. and XIV.	Partitioning, complex formation (PA, TNF), LCC, UV, multiple crystallizations, derivatization, m.p., m.m.p.	9,10
1957	Paper chromatography of carcinogenic hydrocarbons	PC	254
1959	The role of higher polycyclic hydrocarbons in tobacco carcinogenesis	PC, sublimation, m.p., m.m.p.	15
1959	Use of the fine structure of the fluorescence spectrum of 3:4-benzpyrene to increase the certainty of its detection	Fluorescence spectrophotometry	274
	Fluorescence spectral analysis of the products of tobacco smoke		275
1960	Characterization and level of 3,4-benzpyrene by luminescence spectrophotometry at –190 ˚C	Spectrophotometry	287
1961	A method for the estimation of 3,4-benzpyrene in tobacco smoke condensate	Partitioning, PC, LCC, UV	299
1962	A new method for the determination of 3,4-benzpyrene in tobacco smoke condensate	Partitioning, LCC, PC, UV fluorescence	194
1963	A rapid analytical technique for routine determination of benzo[*a*]pyrene in cigarette smoke condensate	LCC, UV	195
1963	A new method for the determination of 3,4-benzpyrene in tobacco smoke condensate	PC	316
1964	Demonstration of carcinogenic hydrocarbons, with special reference to paper chromatography	PC	21
1964	Improved method for the determination of benzo[*a*]pyrene in cigarette smoke condensate	TLC	196
1964	Contribution to the determination of 3,4-benzpyrene in tobacco smoke condensate	LCC, PC	328
1965	Determination of benzo[*a*]pyrene and related compounds in cigarette smoke	PC, TLC, GC, ^{14}C-B[*a*]P	85
1965	Improved methods for determination of benzo[*a*]pyrene in cigarette smoke	LCC, GC, TLC, fluorescence	178
1965	Thin-layer chromatographic separation of benzo[*a*]pyrene from cigarette tar	TLC	197
1965	Contribution to the determination of benzo[*a*]pyrene in tobacco smoke condensate	LCC, TLC, UV absorption and fluorescence	198
1966	The fluorometric determination of benzo[*a*]pyrene in cigarette smoke condensate	Fluorometry	199
1966	The fluorometric determination of 3,4-benzpyrene in low-temperature carbonization and smoke condensates	Fluorometry	339
1968/1970	Gas chromatography of polynuclear aromatic hydrocarbons	GC	345
1971	Rapid thin-layer method for fluorometric determination of benzo[*a*]pyrene in cigarette smoke	LCC, TLC, fluorometry	155
1971/1972	Determination of benzo[*a*]pyrene in smoke condensate by ultraviolet spectroscopy	UV	200
	Modification of routine thin-layer method for determining benzo[*a*]pyrene in smoke	TLC	200
1972	A rapid method for the determination of polycyclic hydrocarbons in cigarette smoke	LCC, PC or TLC, fluorescence	201
1973	Separation analysis of polycyclic aromatic hydrocarbons by high pressure liquid chromatography; elective separation system for the quantitative estimation of isomeric benzpyrenes and coronene	HPLC	365
1976	A rapid method for the determination of benzo[*a*]pyrene . . . in cigarette smoke; Quantitative determination of benzo[*a*]pyrene in cigarette smoke condensates by high pressure liquid chromatography	HPLC	96 203
1976	Analysis of benzo[*a*]pyrene in cigarette smoke	HPLC	204
1976	Gas chromatographic quantitation of polynuclear aromatic hydrocarbons in tobacco smoke	GC	97
1976/1978	. . . polynuclear aromatic hydrocarbons in cigarette smoke condensate . . . . . . multialkylated polynuclear aromatic hydrocarbons incigarette smoke condensate . . .	GelC, GC, HPLC, GC/MS	16, 17, 18 19
1977	Analysis of polycyclic aromatic hydrocarbons (PAH) in the condensates of natural and synthetic smoking materials	GelC, TLC, ^{14}C-labelled B[*a*]P (and DB[*a,h*]A)	179
1981	Analysis of benzo[*a*]pyrene in cigarette smoke by high-performance liquid chromatography	HPLC	204
1986	. . . determination of benzo[*a*]pyrene . . . in the total particulate matter of cigarette smoke by high-performance liquid chromatography	HPLC	100
1988/1990	The determination of benzo[*a*]pyrene in the total particulate matter of cigarette smoke	HPLC	205, 206
1989	An alternative isolation procedure for the subsequent determination of benzo[*a*]pyrene in total particulate matter of cigarette smoke	HPLC	207

Table 6 (*cont.*)

Date	Titles	Methodologies [a]	Reference
1998	The measurement of benzo[*a*]pyrene in mainstream cigarette smoke	HPLC, Sep-Pak® cartridge	208
1999	Determination of benzo[*a*]pyrene in complex matrix by multidimensional high-performance liquid chromatography	HPLC	209
1999	Quantitation of benzo[*a*]pyrene from mainstream smoke by liquid chromatography tandem mass spectrometry	LCC/MSS	210
2002	Determination of benzo[*a*]pyrene in cigarette smoke total particulate matter by two-dimensional chromatography	2-DimC	211
2004	CORESTA issued its description of a GC/MS method for the quantitative determination of B[*a*]P in CSC	GC/MS	401

[a] Abbreviations: 2-DimC = two-dimensional chromatography; GelC = gel chromatography; GC = gas chromatography; HPLC = high pressure liquid chromatography; LCC = liquid column chromatography; m.p. = melting point; m.m.p. = mixture melting point; MS = mass spectrometry; PA = picric acid; PC = paper chromatography; TLC = thin layer chromatography; TNF = 2,4,7-trinitrofluorenone.

results obtained on these PAHs alone and the same PAHs identified in MSS and CSC. As compounds studied individually as reactants in biological assays (*in vitro* and *in vivo*), the following information was generated: B[*a*]A is at best only a very weak skin tumorigen, if tumorigenic at all (28). B[*a*]A is mutagenic in the Ames test (379, 380, 384). DMB[*a*]A, DB[*a,h*]A, and B[*a*]P are carcinogenic to rodent skin, sarcogenic on subcutaneous injection (31, 32, 423), and all are mutagenic in the Ames test (379, 380, 384). All four PAHs have been reported in cigarette MSS as part of a complex mixture of possibly 100000 entities of which about 4800 have been identified, including over 500 PAHs (1). At ng/cig yields, the levels of these four PAH components in MSS are extremely low. Unlike the testing of individual compounds, the biological evaluation of complex mixtures such as MSS and CSC presents unique problems in defining the causative agent for a biological response. Much of the history of controversy surrounding the assessment of the biological activity of MSS and CSC has been in determining the substance(s) or family of compounds effecting the biological activity (or lack thereof). Contrary to the opinions of some scientists and scientific bodies, one cannot extrapolate the biological activity of an individual compound to that same compound in a complex mixture such as MSS or CSC (391, 424–426, 431). Although both MSS and CSC contain these four PAHs and in certain circumstances CSC has been shown to induce tumors in mouse skin-painting studies, mice (regardless of genetic modifications or susceptibilities) are not men. And therein lies the problem. The mixtures known as MSS and CSC are so complex that it is not possible to ascribe their biological activity to any individual component because of the known behavior of that component when administered individually. It is also not possible to take a "leap of faith" in extrapolating mouse skin-painting results to humans. Only recently has the concept of complex mixtures in relation to the understanding of the complexity of carcinogenesis taken hold (431). Perhaps the reason why MSS is less tumorigenic than expected in laboratory animals or humans is because of the presence of other MSS or CSC components that are known to inhibit or prevent the tumorigenicity to mouse skin of the four PAHs discussed (55). While it may be contentious to some, we consider that the bulk of the evidence available to date indicates the following: There has never been an unequivocal proof of human carcinogenesis directly attributed to any of these four PAHs from cigarette smoking. When one reviews the history of these four PAHs in MSS or CSC, it is obvious that many unanswered questions remain.

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