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# Toxic Chemicals in Cigarette Mainstream Smoke – Hazard and Hoopla*

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SUMMARY

These are curious times. The Canadian government has passed legislation that requires cigarette manufacturers to routinely test and publish the amounts of 44 toxic substances in cigarette mainstream smoke (MSS). Following in the footsteps of their northern neighbor, various US legislators and regulators are considering modifications to their cigarette testing and reporting programs that will also list toxicants in MSS. Across the Atlantic Ocean, the European Commission has passed a directive that may also follow the North American lead for public disclosure of MSS toxic chemicals for each brand of cigarette sold in the marketplace. United Kingdom authorities have also expressed their intention to follow this mandate.

It is difficult to understand the motivation and value of these existing or potentially forthcoming legislative actions. Although there is nearly total agreement among the world's scientists that cigarette smoking is a health hazard, few are bold enough to say with credibility which smoke chemicals or classes of chemicals are responsible for the adverse

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effects. Therefore, if the specialists are unable to interpret the smoke toxicant data, how is the general public to use their newfound knowledge?

The posting of smoke chemical toxicant data is also problematic for the Tobacco Industry for several reasons. First, no standard analytical methods exist for most suspected toxicants. Second, the listing of smoke toxicant yields may ignite a 21st Century version of the "tar" wars in the USA during the 1960s; we have already seen evidence of such competition beginning in the US. Third, and most important of all, no one knows whether or not reducing the yield of one or more publicized MSS toxicant will result in a "less hazardous" cigarette.

Assuming that the current situation is approximately as described above, the authors of this paper critically examined the existing lists of MSS toxicants. They discarded chemicals that are no longer relevant, e.g., DDT, *N*-nitrosodiethanolamine, added known smoke constituents that are glaringly absent, e.g., dioxins, and replaced the existing 1950–60s era nonfiltered cigarette MSS yields with those more representative of the present-day marketplace. Data for the Kentucky reference 1R4F cigarette smoked under standardized smoking conditions, i.e., those established by the International Organization for Standardization (ISO) and the Federal Trade Commission (FTC), are used as a surrogate for the modern-day cigarette whenever possible.

A list of smoke toxicants and their approximate concentrations in today's cigarettes is nearly useless without an appropriate ranking of their relative toxicity. Unfortunately, the toxicological data for ranking importance are available for fewer than 5% of the approximately 4800 reported smoke constituents. Although neither of this paper's authors presumes to be a toxicologist, we cite in our discussion several published attempts at ranking smoke toxicants. Specifically, ranking by US Occupational Safety and Health Administration (OSHA) permissible workplace exposure levels, use of US Environmental Protection Agency (EPA) toxicity criteria supplemented with California EPA criteria, and use of the Human Exposure – Rodent Potential methodology and database developed by AMES *et al.* when data are available. There appears to be a wide divergence in the permissible exposures allowable in the workplace and those advocated by environmental regulators. Thus, it is expected that rankings such as those presented herein will ultimately form the basis of MSS toxic chemical prioritization for either attempts at reduction by product developers or development of standardized analytical methods.

This review of MSS toxicants also explores the limitations of toxicological evaluations. The toxicity data used in the above ranking are derived wholly from studies of pure compounds. It is highly improbable that extrapolation of bioassay results determined on an individual compound to that compound when it is a component of a mixture as complex as cigarette MSS is valid. For example, several decades of research involving numerous investigators reported that the benzo[*a*]pyrene (B*a*P) content of cigarette smoke condensate (CSC) accounts for only a few percent of the tumor-bearing animals in the skin-painting bioassay. Subsequently they asserted that the tumorigenic polycyclic aromatic hydrocarbons (PAHs) in CSC could account for no more than 3 to 4% of the tumor-bearing animals. Inclusion of promoters, e.g., phenols, raises the level to about 5% . However, several of the same investigators recently claimed that B*a*P is one of two smoke components responsible for lung cancer in cigarette smokers.

While much is written about the hundred or so toxic components in cigarette smoke, little is published about the numerous nontoxic smoke components that have been shown in various bioassays to counteract the effects of the toxic ones. In some cases the inhibiting components are also listed as toxic, e.g., nicotine inhibits the mutagenicity of *N*-nitrosodimethylamine; the promoter phenol inhibits the tumorigenicity of B*a*P; the weakly tumorigenic benz[*a*]anthracene negates the potent tumorigenicity of B*a*P. On a one-to-one molar basis, many bicyclic, tricyclic, and tetracyclic nontumorigenic PAHs counteract the tumorigenicity of B*a*P and dibenz[*a*,*h*]anthracene.

To further illustrate this murky toxicological situation, the history and current knowledge of the importance of tobacco-specific nitrosamines (TSNAs) to the hazards of smoking is reviewed. In brief, these compounds were discovered in tobacco products and found to transfer to MSS (and sidestream smoke). Toxicological evaluations on the pure compounds demonstrated that they are potent carcinogens. Some public health scientists believed that if the levels of TSNAs could be reduced or lowered in MSS, then this would lead to a "less hazardous" cigarette. Once given this assignment, agronomists discovered that at least for flue-cured tobaccos, the levels of TSNAs can be greatly reduced through the use of indirect heating in the curing barns. This was wonderful news. However, toxicologists soon conducted experiments comparing the toxicity of MSS from flue-cured cigarettes containing high and ultra-low concentrations of TSNAs. It must have been a surprise to these investigators when they could find no significant difference between the toxicities of the two smokes.

Some public health scientists have asserted that the reduction of the per cigarette "tar" delivery below 15 mg/cig does not reduce the risk from smoking because of the hazard resulting from the higher levels of additives used to maintain consumer acceptability. Although no data in support of this assertion have ever been offered, much data generated during the past decade contradict the assertion. Ingredient addition at the usual level or at levels several times greater than normal does produce some minor changes in the smoke chemistry, but these changes do not result in any adverse biological response as measured in various bioassays to determine mutagenicity, tumorigenicity, etc.

From our review of the literature gathered to prepare this paper, we have come to several conclusions. These include the following:

1 It is possible to prepare a list of the known toxicants in MSS and to prioritize some of them based upon existing biological data. However, for more than 95% of the known constituents in MSS, there are no biological data.
2 Even if there were biological data for most MSS components, extrapolation of this pure-compound knowledge to the biological properties of a mixture containing them is beyond our scientific ability.
3 At our current state of scientific knowledge, no one will ever be able to legitimately claim the development of a "less hazardous" cigarette based solely on the reduction of known toxic chemicals in MSS.

4 The approach of reducing “tar” yields of cigarettes appears in retrospect to be the most practical means of producing a “less hazardous” cigarette, because when product developers reduce “tar”, both the known and unknown toxicants are reduced.
5 The ranked toxicants in MSS contain both gas-phase and semi-volatile constituents that appear to be important determinants of toxicity. Some of these constituents, e.g., *N*-nitrosodimethylamine, phenols, are reduced by triacetin-plasticized cellulose acetate filters. These filters also reduce “tar”. Additionally, it is well known that charcoal-containing filters have a high efficiency for removing carbonyl compounds from MSS. Development of more consumer-acceptable products that reduce gas-phase toxicants appears to be another route to a “less hazardous” cigarette.

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## ZUSAMMENFASSUNG

Heutzutage passieren erstaunliche Dinge. Die kanadische Regierung hat ein Gesetz verabschiedet, dass Zigarettenproduzenten verpflichtet, routinemäßig das mengenmäßige Vorkommen von 44 toxischen Substanzen im Hauptstromrauch (HSR) von Zigaretten zu untersuchen und zu publizieren. Verschiedene gesetzgeberische und regulative Instanzen in den USA treten in die Fußstapfen ihres nördlichen Nachbarn und ziehen Änderungen bei Zigarettentests und in der Publikation der Ergebnisse in Betracht, einschließlich der Veröffentlichung toxischer Substanzen im HSR. Auf der anderen Seite des Atlantiks hat die Europäische Kommission eine Direktive verabschiedet, die auch der nordamerikanischen Vorgabe folgen könnte und Informationen über toxische Substanzen jeder einzelnen im Handel erhältlichen Zigarettenmarke der Öffentlichkeit zugänglich gemacht werden müßten. Auch die verantwortlichen Behörden in Großbritannien haben ihre Absicht bekundet, diesem Mandat zu folgen.

Es ist schwierig, die Motive und den Wert dieser bereits existierenden oder möglichen zukünftigen legislativen Schritte zu verstehen. Obwohl Wissenschaftler auf der ganzen Welt fast ausnahmslos darin übereinstimmen, dass Zigarettenrauchen eine Gefährdung für die Gesundheit darstellt, gibt es nur wenige, die kühn genug sind, mit Glaubwürdigkeit zu sagen, welche chemischen Substanzen oder Substanzklassen für die schädigenden Wirkungen verantwortlich sind. Wenn also Spezialisten nicht dazu in der Lage sind, toxische Rauchdaten zu interpretieren, wie wird dann die Öffentlichkeit ihr neu gefundenes Wissen nutzen? Die Aufstellung der toxikologischen Daten für Rauchbestandteile ist auch für die Tabakindustrie aus mehren Gründen problematisch. Erstens gibt es für die meisten verdächtigen toxischen Substanzen keine standardmäßigen analytischen Nachweismethoden. Zweitens könnte eine Rangliste des Gehalts toxischer Substanzen im Rauch eine Variante des 21. Jahrhunderts der Kondensatkriege der 1960er Jahre in den USA entfachen. Es gibt in den USA schon Anzeichen einer beginnenden derartigen Auseinandersetzung. Drittens und von besonderer Bedeutung ist aber, dass niemand weiß, ob die Verringerung des Gehalts einer oder mehrerer publizierter toxischer Substanzen im HSR überhaupt zu einer weniger schädlichen Zigarette führt.

Unter der Annahme, dass die gegenwärtige Situation ungefähr so ist wie oben beschrieben, haben die Autoren dieser Arbeit die existierenden Listen toxischer Substanzen im HSR kritisch untersucht. Dabei wurden Substanzen ausgenommen, die nicht länger von Bedeutung sind, wie z.B. DDT, *N*-Nitrosodiethanolamine, bekannte Rauchinhaltsstoffe, die offenkundig nicht aufgeführt sind, wie z.B. Dioxine, wurden hinzugefügt und die existierenden HSR Werte von Zigaretten ohne Filter aus den1950er und 1960er Jahren wurden durch Werte ersetzt, die für den gegenwärtigen Zigarettenmarkt repräsentativer sind. Werte der 1R4F Kentucky Referenzzigarette, die unter Standardbedingungen gemäß ISO (International Organization for Standardization) und FTC (Federal Trade Commission) Richtlinien abgeraucht wurde, wurden wenn möglich stellvertretend für die heutige marktübliche Zigarette angeführt.

Eine Liste toxischer Substanzen im Rauch und deren annähernde Mengen in heutigen Zigaretten ist ohne geeignetes Ranking ihrer relativen Toxizität weitgehend nutzlos. Leider stehen toxikologische Daten für ein Ranking nur für weniger als 5% der ungefähr 4800 bekannten Rauchkomponenten zur Verfügung. Obwohl keiner der Autoren dieser Arbeit sich anmaßt, Toxikologe zu sein, werden in der Diskussion mehrere publizierte Versuche zitiert, ein Ranking der Toxizität des Rauches vorzunehmen. Hierzu zählen insbesondere das Ranking der Occupational Safety and Health Administration (OSHA) in den USA zur erlaubten Höchstmenge von Substanzen am Arbeitsplatz, die Toxizitätskriterien der Environmental Protection Agency (EPA) in den USA mit dem Zusatz der in Kalifornien geltenden EPA Kriterien sowie die Übertragbarkeit von Tierversuchsdaten auf den Menschen und die von Ames *et al.* entwickelte Datenbank existierender Daten. Es scheint ein großer Unterschied zwischen zulässigen Höchstmengen am Arbeitsplatz und den von Umweltschutzbehörden empfohlenen Höchstmengen zu geben. Es ist demzufolge zu erwarten, dass derartige Rankings letztendlich dazu führen, dass bestimmte toxische Substanzen besondere Berücksichtigung finden, und zwar bei den Produzenten hinsichtlich einer Reduzierung dieser Substanzen als auch bei der Entwicklung analytischer Standardmethoden.

In dieser Übersicht über toxische Substanzen im HSR werden ebenfalls die Grenzen toxikologischer Bewertungen untersucht. Die Toxizitätsdaten der oben genannten Rankings beziehen sich alle auf Studien mit Einzelsubstanzen. Es ist höchst unwahrscheinlich, dass die Ergebnisse aus Tierversuchen mit Einzelsubstanzen extrapoliert werden können, wenn diese Substanz in einem so komplexen Gemisch wie dem HSR einer Zigarette vorliegt. So wurde zum Beispiel in vielen Studien der vergangenen Jahrzehnte berichtet, dass der Benzo[*a*]pyren (B*a*P) Gehalt im Kondensat von Zigarettenrauch (CSC) nur in wenigen Prozent der tumorigenen Wirkung auf der Haut verantwortlich sei. Daraus wurde gefolgert, dass die Tumor verursachenden polyzyklischen aromatischen Kohlenwasserstoffe (PAHs) im CSC für nicht mehr als 3% bis 4% der Tumore auf der Haut von Versuchstieren verantwortlich sein könnten. Das Hinzufügen von Promotoren, wie z.B.

der Phenole, erhöhe die Quote auf bis zu 5%. Einige derselben Forscher haben jedoch kürzlich behauptet, dass B*a*P eine von zwei Substanzen sei, die bei Rauchern Lungenkrebs verursache.

Während über die ungefähr hundert toxischen Substanzen im Zigarettenrauch viel geschrieben wurde, wurde über die zahlreichen nichttoxischen Rauchsubstanzen, die in vielen Tierversuchen nachweislich den Wirkungen der toxischen Substanzen entgegenwirken, wenig berichtet. In einigen Fällen werden die inhibitorischen Komponenten ebenfalls unter den toxischen Substanzen gelistet, so hemmt z.B. Nikotin die Mutagenität von *N*-Nitrosodimethylamine, der Promotor Phenol hemmt die Tumor verursachende Wirkung von B*a*P, das schwach Tumor verursachende Benz[*a*]-anthracen macht die starke tumorigene Wirkung von B*a*P zunichte. Auf einer eins zu eins molaren Basis wirken viele bizyklische, trizyklische und tetrazyklische nicht tumorigene PAHs der tumorigenen Wirkung von B*a*P und Dibenz[*a*,*h*]anthracene entgegen.

Um die unklare toxikologische Situation näher aufzuzeigen, wird ein Überblick über die Geschichte und das gegenwärtige Wissen zur Bedeutung tabakspezifischer Nitrosamine (TSNAs) für die Risiken des Rauchens gegeben. Kurz gesagt, wurden diese Substanzen in Tabakprodukten entdeckt und es wurde festgestellt, dass sie in den HSR (und Nebenstromrauch) übergehen. Toxikologische Beurteilungen der reinen Substanzen haben gezeigt, dass es sich um starke Karzinogene handelt. Einige Wissenschaftler des Öffentlichen Gesundheitswesens vertraten die Ansicht, dass die Verringerung der TSNA Mengen im HSR zu einer „weniger schädlichen" Zigarette führen würde. Nach dieser Festlegung haben Agrarwissenschaftler entdeckt, dass die TSNA Mengen zumindest bei flue-cured Tabaken durch die Verwendung indirekter Heizsysteme in den Trockenschuppen stark verringert werden können. Dieses waren wunderbare Neuigkeiten. Toxikologen führten bald Untersuchungen durch, in denen die Toxizität des HSR von flue-cured Tabaken mit hohen und sehr niedrigen TSNA Konzentrationen miteinander verglichen wurde. Diese Forscher müssen überrascht gewesen sein festzustellen, dass in der Toxizität des Rauches beider Tabake kein signifikanter Unterschied bestand.

Einige Wissenschaftler des Öffentlichen Gesundheitswesens haben behauptet, dass die Verringerung des Kondensatgehalts pro Zigarette unter 15 mg das mit dem Rauchen verbundene Risiko nicht vermindert, da die erhöhte Zugabe von Additiven zur Aufrechterhaltung der Akzeptanz des Rauchers eine Gesundheitsgefahr darstelle. Während in der Vergangenheit bisher keine Daten präsentiert wurden, die diese Behauptung stützen würden, wurden im vergangenen Jahrzehnt viele Ergebnisse erhalten, die dieser Behauptung widersprechen. Die Zugabe von Zusatzstoffen in gewöhnlicher oder mehrfach erhöhter Menge führt zu leichten Veränderungen in der Rauchchemie, diese Veränderungen haben jedoch, wie in verschiedenen Tierversuchen nachgewiesen, in denen die Mutagenität oder Tumorigenität usw. untersucht wurde, keine nachteiligen biologischen Reaktionen zur Folge.

Aus unserer Übersicht der Literatur kommen wir zu folgenden Schlussfolgerungen:

1 Es ist möglich, eine Liste der bekannten toxischen Substanzen im HSR zusammenzustellen und einige dieser Substanzen aufgrund existierender biologischer Daten als besonders toxisch zu klassifizieren. Für mehr als 95% der bekannten Komponenten des HSR sind jedoch keine biologischen Daten bekannt.
2 Auch wenn es biologische Daten für die meisten Inhaltsstoffe des HSR gäbe, wäre eine Extrapolation der toxischen Eigenschaften der Einzelsubstanzen auf die biologischen Eigenschaften eines Gemisches, das diese Substanzen enthält, außerhalb unserer wissenschaftlichen Fähigkeiten.
3 Auf der Basis unserer heutigen wissenschaftlichen Kenntnisse wird niemand berechtigterweise die Entwicklung einer „weniger schädlichen Zigarette" fordern können, die auf einer alleinigen Reduzierung bekannter toxischer Substanzen im HSR beruht.
4 Der Ansatz, den Kondensatgehalt von Zigaretten zu reduzieren, erscheint rückschauend betrachtet der praktikabelste Weg zur Herstellung einer „weniger schädlichen" Zigarette zu sein, weil bei einer Verringerung des Kondensatgehalts durch den Produzenten sowohl die bekannten als auch die unbekannten toxischen Substanzen reduziert werden.
5 Das Ranking toxischer Substanzen im HSR enthält sowohl Gasphasen- als auch semivolatile Substanzen, die für die Toxizität von entscheidender Bedeutung zu sein scheinen. Einige dieser Komponenten, z.B. *N*-Nitrosodimethylamin und die Phenole werden durch mit Triacetin behandelten Zelluloseacetatfiltern reduziert. Diese Filter verringern ebenfalls den Kondensatgehalt. Außerdem ist bekannt, dass Carbonylverbindungen durch Aktivkohlefilter sehr wirksam aus dem HSR entfernt werden. Die Entwicklung von mehr Produkten, die vom Konsumenten akzeptiert werden und bei denen toxische Substanzen in der Gasphase reduziert sind, scheint ein weiterer Weg zu einer „weniger schädlichen" Zigarette zu sein.

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RESUME

Les temps sont curieux. Le gouvernement canadien a établi une loi exigeant des tests réguliers et la publication des teneurs de 44 substances toxiques dans la fumée du courant principal de la cigarette (CP) par les producteurs de cigarettes. Suivant l'exemple de leur voisin du Nord, les législateurs et l'administration des Etats Unis envisagent de modifier les tests sur les cigarettes et les méthodes d'information, exigeant également l'évaluation des substances toxiques du CP. De l'autre côté de l'océan atlantique, la Commission Européenne a soumis une directive qui pourrait également suivre l'exemple de l'Amérique du Nord, en exigeant de rendre public les substances toxiques du CP pour toutes les marques de cigarettes commercialisées. Les autorités du Royaume Uni ont également exprimé leur intention de suivre ce mandat.

Il est difficile de comprendre la motivation et la valeur de ces actions législatives potentielles ou ultérieures. Bien qu'il y ait presque un accord unanime entre les chercheurs du monde entier que fumer des cigarettes présente un risque sanitaire pour le fumeur, peu peuvent affirmer avec crédibilité, quels sont les composés chimiques, ou classes de composés, de la

fumée responsable d'effets néfastes sur la santé. Par conséquent, si même les spécialistes ne sont pas capables d'interpréter clairement les données toxiques de la fumée, comment le public va-t-il utiliser ces nouvelles informations ?

La compilation des données sur les composants toxiques de la fumée pose également des problèmes à l'industrie du tabac pour plusieurs raisons. Premièrement, il n'y a pas de procédures analytiques standard pour la plupart des composants supposés être toxiques. Deuxièmement, la compilation des teneurs en substances toxiques pourrait provoquer une variante du 21ième siècle de la « guerre du goudron » des années 1960 aux Etats Unis ; nous avons déjà observé l'existence d'une telle compétition démarrant aux Etats Unis. Troisièmement, et ce qui importe avant tout, personne ne sait si la réduction de la teneur en un seul ou plusieurs composants toxiques du CP produira une cigarette « moins dangereuse ».

En supposant que la situation actuelle se présente environ comme décrit ci-dessus, les auteurs de cette revue ont étudié à fond les listes existantes sur les composants toxiques du CP. Ils ont exclu les composants qui ne sont plus pertinents, tels que le DDT et le *N*-nitrosodiethanolamine, ajouté des composants connus qui sont manifestement absents, tels que la dioxine, et ont substitué les teneurs du CP de cigarettes sans filtre des années 1950–60 avec les données de cigarettes plus représentatives du marché actuel. Les données de la cigarette de référence 1R4F, fumée sous les conditions normalisées ISO (International Organization for Standardization) et FTC (Federal Trade Commission) sont utilisées quand cela est possible comme substitut de la cigarette actuelle.

Une liste des composants toxiques et leurs concentrations approximatives dans les cigarettes commercialisées est pratiquement inutile sans évaluation appropriée de la toxicité relative de chaque composant. Malheureusement, les données toxiques permettant une évaluation ne sont disponibles que pour moins de 5% seulement des 4800 composants environ rapportés. Bien que les auteurs ne présument pas être toxicologistes, ils rapportent dans la discussion sur plusieurs essais publiés de l'évaluation des composants toxiques de la fumée. En particulier, l'évaluation de l'exposition maximale sur le lieu de travail établie par l'Occupational Safety and Health Administration (OSHA) aux Etats Unis, l'utilisation des critères toxicologiques de l'Environmental Protection Agency (EPA) aux Etats Unis avec en supplément des critères de l'EPA en Californie, l'utilisation de la méthodologie exposition humaine – potentiel chez les rongeurs, et, si des données sont disponibles, base de données développée par AMES *et al*. La divergence entre les expositions admissibles sur le lieu de travail et les concentrations recommandées par des réglementations de l'environnement semble être grande. Ainsi, il est attendu que de telles évaluations attirent une attention particulière sur des composants chimiques particuliers, soit en vue d'une réduction de la part du producteur, soit en vue d'un développement de méthodes analytiques standardisées.

Cette revue des composants toxiques du CP examine également les limites d'évaluations toxicologiques. Toutes les données toxiques utilisées dans l'évaluation ci-dessus ont été obtenues par des études de substances uniques. Il est particulièrement improbable qu'une extrapolation des résultats obtenus d'une substance unique par des tests biologiques puisse être faite à la même substance, si cette substance est la composante d'un mélange aussi complexe que le CP. Au cours de plusieurs décennies, de nombreux chercheurs ont rapporté que la teneur en benzo[*a*]pyrène (B*a*P) du condensat de fumée de cigarette (CSC) explique quelques pourcentages seulement des formations tumorales chez les animaux dans les tests biologiques, comprenant l'application d'un composant sur la peau des animaux. Par la suite, ils ont affirmé que les hydrocarbures polycycliques aromatiques (PAH) tumorigènes du CSC ne pouvaient pas être responsables que de plus de 3% ou 4% des formations tumorales chez les animaux. L'inclusion de promoteurs, tels que les phénols, augmente le taux à environ 5%. Cependant, quelques-uns de ces mêmes chercheurs ont récemment prétendu que le B*a*P est un des deux composants de la fumée responsable du cancer du poumon chez les fumeurs.

Tandis que beaucoup a été publié sur à peu près cent composants toxiques de la fumée de cigarette, il y a rarement de publications sur les nombreux composants non-toxiques, qui ont montré un effet d'inhibition des composants toxiques dans plusieurs tests biologiques. Dans certains cas, les composants inhibiteurs figurent également parmi les composants toxiques, la nicotine inhibe par exemple la mutagénicité de *N*-nitrosodiméthylamine ; le promoteur phénol inhibe la tumorigénicité de B*a*P ; le benz[*a*]anthracène, légèrement tumorigène, annule la forte tumorigénicité de B*a*P. Sur une base molaire « one-to-one », plusieurs PAH bicycliques, tricycliques et tetrecycliques non-tumorigènes inhibent la tumorigénicité de B*a*P et dibenz[*a,h*]anthracène.

Pour illustrer cette situation toxicologique, l'historique et la connaissance actuelle sur l'importance des nitrosamines spécifiques du tabac (TSNA) pour les risques sanitaires du fumage sont passés en revue. En bref, ces composants ainsi que leur transfert dans le CP (et le courant secondaire) ont été découverts dans les produits du tabac. Les évaluations toxicologiques des substances uniques ont montré qu'il s'agit de carcinogènes puissants. Certains chercheurs de la santé publique ont supposé que si les teneurs en TSNA du CP pouvaient être réduites, cela devrait permettre d'obtenir une cigarette « moins nocive ». Après cette déclaration, les agronomes ont découvert que les teneurs en TSNA peuvent être réduites par l'utilisation d'un chauffage indirect dans les séchoirs. Ces nouvelles étaient merveilleuses. Cependant, des toxicologistes ont vite mené des essais pour comparer la toxicité du CP de cigarettes « flue-cured » ayant des teneurs élevées et ultra-réduites en TSNA. Cela a été une surprise pour ces chercheurs de trouver qu'il n'y avait pas de différence significative de la toxicité des deux fumées.

Certains chercheurs de la santé publique ont affirmé que la réduction de la teneur en goudron par cigarette en dessous de 15 mg/cig ne réduit pas le risque engendré par la cigarette à cause du danger résultant de l'apport plus élevé d'additifs pour sauvegarder l'acceptabilité par le consommateur. Tandis que cette assertion n'a jamais été confirmée par des résultats, beaucoup de données obtenues sont en contradiction avec cette assertion. L'apport d'ingrédients, à des niveaux habituels ou plus élevés, entraîne des changements insignifiants dans la chimie de la fumée, mais, comme cela a été mesuré dans plusieurs tests biologiques pour déterminer la mutagénicité, tumorigénicité, etc., ces

changements ne conduisent pas à des réactions biologiques négatives.

La littérature examinée pour préparer cette revue nous mène à tirer les conclusions suivantes :

1 Il est possible de préparer une liste des composants toxiques connus du CP et de prêter une attention particulière à certains d'entre-eux par rapport aux données biologiques. Cependant, pour plus de 95% des composants du CP, il n'existe pas de données biologiques.
2 Même si des données biologiques existaient pour la plupart des composants du CP, l'extrapolation des données obtenues des substances uniques aux propriétés biologiques d'un mélange contenant ces substances est au-delà de notre capacité scientifique.
3 D'après nos connaissances scientifiques actuelles, il ne sera jamais possible de prétendre au développement d'une cigarette « moins nocive », basée sur la réduction de composants toxiques connus du CP seulement.
4 L'approche qui consiste en une réduction de la teneur en goudron d'une cigarette semble être la méthode la plus pratique pour arriver à une cigarette « moins nocive », parce qu'en réduisant la teneur en goudron, les composants toxiques à la fois connus et inconnus sont réduits.
5 Les substances évaluées comme toxiques du CP contiennent à la fois des constituants volatils et semi-volatiles, qui semblent être déterminants pour la toxicité. Certains de ces composants, comme le *N*-nitrosodiméthylamine et les phénols sont réduits par des filtres d'acétate de cellulose plastifiés par le triacétine. Ces filtres réduisent également la teneur en goudron. En plus, il est bien connu que des filtres contenant du charbon éliminent efficacement les composés carbonyle de la fumée. Le développement de plus de produits acceptables pour le consommateur qui réduisent les substances toxiques de la phase gazeuse semble être une voie alternative vers une cigarette « moins nocive ».

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## 1 INTRODUCTION

> Things should be made as simple as possible, but not any simpler. — *Albert Einstein*

The quotation from Professor Einstein seems extremely appropriate when embarking on any discussion attempting to link cigarette MSS composition to the hazards of smoking. The simplistic dream of both Tobacco Industry and public health scientists is to identify the smoke constituents responsible for adverse health effects and either greatly reduce or eliminate those chemicals to create "less hazardous" products. Whether or not this hope can be made reality is unknown. However, the authors of this review can state with some certainty that the current status of chemical and toxicological sciences does not allow us to demonstrate that any specific chemicals or classes of chemicals present in tobacco smoke are responsible for the health hazards of smoking. Our current belief is in concert with that expressed earlier by the National Research Council of the National Academy of Sciences (1) regarding the status of knowledge relating smoke composition with health hazards.

> Even after decades of serious investigation, we do not understand the role of tobacco smoke components in producing chronic diseases, such as arteriosclerosis, emphysema and malignant neoplasms. The task of identifying the toxic components is overwhelming and must be considered currently impossible. However, we can identify groups of agents from a knowledge of their chemical similarity to agents generated in a standard control substance.

Soon following the pioneering epidemiological research relating smoking to carcinoma of the lung by WYNDER and GRAHAM in the USA and DOLL and HILL in the UK (2) and the generation of tumors in mice following skin painting with smoke condensate by WYNDER *et al.* (3), chemists have been attempting to answer the question as to what components in tobacco smoke are responsible for the reported findings. Prior to 1954, tobacco smoke was recognized as an extremely complex mixture but very little was known about its composition. Fewer than 100 components had been reported, but many identifications were subsequently shown to be incorrect. As reviewed by GREEN and RODGMAN (4), among the first published lists of tobacco smoke constituents was that of KOSAK (5). His list contained approximately 80 entries of which almost one-half were questionable in regards to correct identity. GREEN and RODGMAN (4) estimated that there are approximately 4800 known components in tobacco smoke.

Lists of toxic components in tobacco smoke are also not new phenomena. Among the first of these lists was one contained in the 1964 report of the ADVISORY COMMITTEE to the US Surgeon General (6) on smoking and health. It seems as though there has been either a new list of MSS toxicants published or a re-publication of a previously published list every year since the first list appeared. Perhaps most notable among the smoke toxicant list publications is the "List of 43" prepared by HOFFMANN and HECHT (7). This list was used by the US Environmental Protection Agency (EPA) (8) to bolster their argument that exposure to second-hand cigarette smoke is a cause of lung cancer in nonsmokers. RODGMAN (9) has pointed out the deficiencies in the "List of 43" and interpretations made by the EPA. Within the last few years, additional and/or revised lists of smoke toxicants have been published by BAKER and PROCTOR (10), HOFFMANN *et al.* (11), HOFFMANN and HOFFMANN (12), and SMITH *et al.* (13–15). Although the cited lists and others not presented herein contain much valuable information, they tend to perpetuate data contained in the earliest publications that are unsupportable, incorrect and irrelevant to the current situation.

Even though the emphasis over the years on all but one class of toxicants has waxed and waned, it has become common practice since the mid-1980s to publish lists of cigarette smoke toxicants and their per cigarette deliveries. With time, the toxicant lists have become longer and longer. Because they are, by definition, tobacco-specific, the TSNAs are the one component class that still remains in the limelight. Interestingly, the identification of PAHs in MSS progressed from a few in the 1950s to more than 500 in the 1970s. That situation differed greatly from the *N*-nitrosamine (NNA) case where little research on additional NNAs in MSS was conducted once the tobacco-specific NNAs were identified.

In several instances, listed toxicants have 1) no identified precursor in tobacco, 2) no quantified MSS levels, 3) a

possible artifactual origin, or 4) an unconfirmed presence. Some cigarette MSS components considered toxic when encountered environmentally, e.g., the dioxins, are not included in any of the cigarette smoke toxicant lists. Also interesting is the fact that some toxicants for which no or only a few quantitative data are available are given equal weight to other toxicants for which literally hundreds of quantitative data have been generated since the mid-1950s, cf. the hundreds of publications on the MSS level of B*a*P vs. the few that only list dibenzo[*a,l*]pyrene or benzo[*b*]furan as *present*.

The oft-repeated assertions [see review by RODGMAN (16)] that ingredients (flavorants, casing materials, humectants) added to tobacco enhance the levels of cigarette MSS toxicants as well as the adverse biological effect of MSS are without merit. No individual or agency making such claims has ever presented detailed data to support such assertions. On the other hand, considerable laboratory evidence has been generated to discount both the adverse compositional and biological assertions (17–23).

Whether or not a chemical constituent is included in the list of smoke toxicants appears to be the result of a haphazard process. There are at least two types of smoke toxicant lists that may be useful. One is a historical record of every toxic component that has been identified, and a second, more useful list related to the development of a "less hazardous" cigarette, would contain all known toxic compounds found in currently-sold commercial cigarette MSS. For both lists, criteria should be stated up front documenting justification for including a smoke constituent. Among these criteria should be the following:

- Confirmation of identity by more than one researcher or research group
- Documentation of carcinogenic properties by the International Agency for Research on Cancer (IARC), US EPA, National Toxicology Program, etc.
- Documentation of other toxic properties by appropriate agencies or scientific studies
- An assessment of the quantification method and the quality of the analytical result. From a risk assessment perspective, qualitative identification of smoke toxicants is practically useless.

As you will note from the title of this paper, the authors have used the term *hoopla* in reference to reports of toxic chemicals in cigarette MSS. *Hoopla* is defined by WEBSTER (24) as "excited commotion". As RICKERT and KAISERMAN have already pointed out (25), "Surveys of Canadian smokers have demonstrated that their [*sic*] continues to be confusion regarding the meaning of the numbers for "tar," nicotine and CO which appear on every package of Canadian cigarettes" (26,27). Because smokers cannot understand "tar" and nicotine labeling, it is difficult to understand how they will interpret toxic compound data. Thus far in antismoking efforts, it appears that at least one of the prime uses of a toxic substance list is the production of public service advertisements. These pronouncements, e.g., the one in 1998 by the National Center for Tobacco Free Kids (28), of the dangers of smoking tend to focus on commonly known toxicants such as arsenic. Rarely mentioned are such substances as B*a*P or 4-(*N*-methylnitrosamino)-1-(3-pyridinyl)-1-butanone (NNK) because the general public has no recognition of these terms.

In recent years a trend to integrate quantitative risk assessment into the listing of smoke toxicants has emerged. These techniques rely on relative toxicity values published by the US Occupational Safety and Health Administration (OSHA), US EPA, IARC, etc. and ultimately allow a relative order of potential harm ranking of the known smoke toxicants. This process has severe limitations among which are the following:

- The smoke yield data for many known/suspected smoke toxicants are of unknown quality. This problem has been exacerbated by the introduction of human smoking conditions into the analytical process. Additionally, few of the reported smoke yields have been determined by validated analytical methods.
- For the reported 4800 smoke constituents, there are existing toxicity data for fewer than 5% of the compounds.
- As RODGMAN (9) has pointed out, mainstream tobacco smoke contains many inhibitors, anticarcinogens, and antimutagens that must be accounted for in assessing the potency of an individual chemical or class of chemicals in cigarette smoke.

and the most significant deficiency of all

- All of the data for smoke toxicants come from animal studies on individual compounds. Prediction of complex mixture toxicology from data on individual components as well as prediction of the toxicology of an individual component in a complex mixture is beyond the current capability of science.

However, if progress is to be made in relating MSS composition to adverse health effects of smoking, quantitative risk assessment of smoke toxicants may be a necessary first step in identifying the relative importance of compounds. Additionally, as SAINT-JALM (29) recently stated concerning the development of validated analytical methods, ". . . there is need to set criteria in order to select which methods should be developed as a priority and it is the intention of CORESTA to work in this direction". Quantitative risk assessment may be a beginning approach to selecting target compounds.

Developing a quantitative risk assessment for MSS toxicants may be beyond the realm of scientific competency for this paper's authors because neither is a degreed or certified toxicologist. However, published relative toxicity assessments by R.J. Reynolds Tobacco Company toxicologists (30), VORHEES *et al*. (31), RICKERT and KAISERMAN (25), TRICKER (32), and FOWLES and BATES (33) serve as our guide in this endeavor.

The study of over forty design technologies to control the delivery and composition of cigarette MSS eventually led to the discovery, development, and use of a few significant ones (34). None of the significant technologies was an outgrowth of various "less hazardous" cigarette activities sponsored by non-Tobacco Industry institutions. All were a product of US Tobacco Industry efforts and were part of commercial cigarette design before the first experiments were conducted in the National Cancer Institute (NCI) Smoking and Health Program on the "less hazardous" cigarette (35). Since the mid-1950s, the use of these technologies in cigarette design either individually or in concert has resulted in the gradual reduction in the levels of many of both the particulate- and vapor-phase toxicants in

cigarette MSS. Unfortunately, the listed per cigarette delivery range of a particular toxicant often includes data collected on the MSS from commercial cigarettes manufactured in the 1950s and 1960s. Thus, the listed range in terms of the deliveries of MSS components from more recently manufactured cigarettes is unrealistic.

The US Tobacco Industry has recently been criticized because it has introduced no significant new cigarette design technology since 1975 (12). Examination of the annual sales-weighted average "tar" yield for US commercial cigarettes [cf. Figure 3 in RODGMAN (34)] reveals that by the late 1960s the 40% to 50% reduction in MSS "tar" yield, i.e., a reduction from 38–39 mg/cig to 19–20 mg/cig, attained and surpassed the goal originally proposed by WYNDER in 1957 to resolve the lung cancer situation (36). Overlooked by the critics is the fact that the eight significant technologies used in concert and to different degrees have resulted in an additional 40% reduction to about 12 mg/cig in the sales-weighted Federal Trade Commission (FTC) "tar" delivery from 1975 to date.

## 2 SOME TOXICANTS IN CIGARETTE MAINSTREAM SMOKE – A 21st CENTURY LIST

The genesis of our cigarette MSS toxicants list contained in Table 1 originates from the private files of the paper's authors. We have also borrowed liberally from smoke toxicant lists cited earlier in this paper, i.e., references 6 through 15. The table contains 149 entries of which we have highlighted eight (in bold font). The highlighted entries are toxicants that continue to be found in many compilations that have either insufficient evidence of their existence in smoke or are components that have been discontinued in tobacco agronomy for decades and appear to be irrelevant to modern cigarettes. In their recent review, BAKER and PROCTOR (10) initiated the practice of specially designating these smoke components and it is our hope that the compounds will disappear from future lists unless their presence is more firmly documented. We have left these compounds in our list so that we can discuss them and, where toxicity data are available, evaluate the relative harm potential of the substance.

Unfortunately, in including compounds in Table 1, we have not followed our own advice of developing specific criteria to either accept or reject a smoke toxicant from the list. This is a large task in itself and would have broadened the scope of our endeavor to expand beyond both the presentation and publication limits of our current assignment. However, we do recommend that CORESTA undertake this criteria-setting approach as a future work item.

As a substitute for criteria setting, we have included in Table 1 four items which influenced selection of smoke toxicants. These (designated by "X" in Table 1) include the following:

- Listing in the 1993 US Consumer Product Toxicity Testing Plan, 19 toxicants
- Listing in the Canadian Government Testing Protocol, 46 toxicants
- Carcinogenicity classification by IARC, 83 toxicants
- Listing in the US EPA tables as hazardous chemicals for Toxic Chemical Release Inventory, 92 toxicants.

Instead of listing the historically determined minimum and maximum yield of a MSS toxicant, we have chosen to use the yield for the Kentucky reference 1R4F cigarette. Many of the yields listed in previous toxicant lists are from nonfiltered cigarettes of 1950s and 1960s vintage. Because more than 90% of today's smokers consume filtered cigarettes, values from nonfiltered cigarettes of a past era are not appropriate for analysis. Table 1 lists five primary sources of cigarette yields. These include 1R4F data from Rickert at Labstat International, Inc., R.J. Reynolds Tobacco Company (RJRT) yields either published or on the ECLIPSE cigarette website, and yields published by Vector Tobacco Company on the OMNI cigarette website. When there were multiple instances of 1R4F yields, the highest value was chosen to be the "Comparison Cigarette Value". If 1R4F data were not available from these sources, yields of INBIFO control cigarettes were chosen as representative of current commercial cigarettes. If none of the previously cited sources had data on listed smoke toxicants, then the maximum value reported by HOFFMANN *et al.* was used for further analysis. And finally, a variety of miscellaneous sources were used to obtain cigarette mainstream yield data, when the five primary sources did not produce results. The references to all the data sources are contained within the Table References. All further analyses that required a cigarette MSS yield used the value in the "Comparison Cigarette Value" column.

## 3 SMOKING-MACHINE YIELDS IN TABLE 1 FOR 1R4F CIGARETTE

Although the authors of this paper are aware of the existing controversy concerning proper analytical smoking-machine methodology for determining "Comparison Cigarette Smoke Yields", e.g., BAKER (37), we chose to use yields generated by the existing US FTC or International Standards Organization (ISO) methods. Among the reasons for this choice are the following:

- There exist few data for yields under alternate smoking regimes.
- There is no agreement as to which alternate smoking regime best represents human smoking.
- Although some public health advocates state that existing standard methods underestimate human smoking yields, no smoking-machine methodology takes into account the actual retention of any smoke analyte.
- In terms of quantitative risk assessment, the science is so crude that orders of magnitude changes in smoke yields are necessary to make a significant difference in the outcome of the analysis.

## 4 OBSOLETE SMOKE TOXICANTS

The only non-tobacco specific nonvolatile *N*-nitrosamine identified in tobacco and tobacco smoke is *N*-nitrosodiethanolamine (NDELA) (IARC, 38). Its presence in tobacco more than two decades ago was related to the use of the sucker growth inhibitor, the diethanolamine salt of maleic hydrazide. Because of a 1981 ban on its use in tobacco agronomy by EPA (39), the diethanolamine salt

**Table 1. Some toxicants in cigarette mainstream smoke.** Agents in **bold** have been included in previous lists of cigarette MSS toxicants, but no longer appear to be relevant. See footnotes for details. **Comparison cigarette values** were chosen in the following order of preference: greatest 1R4F value > INBIFO control > Hoffmann *et al.* max. > miscellaneous source. **Compound names shown in brackets { }** are not the current IUPAC-approved name. "P" indicates a substance present in MSS but is unquantifiable. **IARC categories** include the following: 1 (carcinogenic to humans); 2A (probably carcinogenic to humans); 2B (possibly carcinogenic to humans); 3 (not classifiable as to its carcinogenicity to humans). The following **abbreviations** are used for the *N*-heterocyclic amines (Sugimura compounds): 2-amino-9*H*-pyrido[2,3-*b*]indole (A$\alpha$C); 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeA$\alpha$C); 2-amino-3-methyl-3*H*-imidazo[4,5-*f*]quinoline (IQ); 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1); 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2); 2-amino-6-methyldipyrido[1,2-*a*:3',2'-*d*]imidazole (Glu-P-1); 2-aminodipyrido[1,2-*a*:3',2'-*d*]imidazole (Glu-P-2); 2-amino-1-methyl-6-phenyl-1*H*-imidazo[4,5-*b*]pyridine (PhIP); 2-amino-3,4-dimethyl-3*H*-imidazo[4,5-*f*]quinoline (MeIQ).

| Agent | CAS no. | INBIFO control [a] µg/cig | RJR 1R4F [b,c,d] µg/cig | Omni 1R4F [e] µg/cig | Rickert 1R4F [f] µg/cig | Hoffmann *et al.* [g] max. µg/cig | Misc. source µg/cig | Comparison cig value µg/cig | US CPSC test [h] | Canadian gov't test [i] | IARC class [j,k,l] | EPCRA sect. 313 [m] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Polynuclear aromatic hydrocarbons* | | | | | | | | | | | | |
| Acenaphthene | 83-32-9 | 4.68E−02 | | | 3.77E−02 | | | 3.77E−02 | | | | |
| Acenaphthylene | 208-96-8 | 6.43E−02 | | | 7.25E−02 | | | 7.25E−02 | | | | |
| Anthracene | 120-12-7 | 3.51E−02 | | | 4.31E−02 | | | 4.31E−02 | | | | X |
| Benz[*a*]anthracene | 56-55-3 | 1.01E−02 | 1.05E−02 | | 1.24E−02 | 7.00E−02 | | 1.24E−02 | | | 2A | X |
| Benz[*e*]acephenanthrylene {benzo[*b*]fluoranthene} | 205-99-2 | | | | 5.50E−03 | 2.20E−02 | | 5.50E−03 | | | 2B | X |
| Benzo[*a*]pyrene | 50-32-8 | 5.10E−03 | 5.40E−03 | 4.58E−03 | 5.70E−03 | 4.00E−02 | | 5.70E−03 | X | X | 2A | X |
| Benzo[*c*]phenanthrene | 195-19-7 | | | | | | | | | | 3 | |
| Benzo[*e*]pyrene | 192-97-2 | | | | 3.90E−03 | 4.00E−02 | | 3.90E−03 | | | 3 | |
| Benzo[*ghi*]perylene | 191-24-2 | 1.73E−03 | | | 1.10E−03 | | | 1.10E−03 | | | | X |
| Benzo[*j*]fluoranthene | 205-82-3 | | | | | 2.10E−02 | | 2.10E−02 | | | 2B | X |
| Benzo[*k*]fluoranthene | 207-08-9 | | | | 1.30E−03 | 1.20E−02 | | 1.30E−03 | | | 2B | X |
| Benzo[*rst*]pentaphene {dibenzo[*a*,*l*]pyrene} | 189-55-9 | | | | | 3.20E−03 | | 3.20E−03 | | | 2B | X |
| Chrysene | 218-01-9 | 1.44E−02 | | | 1.36E−02 | | | 1.36E−02 | | | 3 | X |
| Chrysene, 5-methyl- | 3697-24-3 | 7.60E−03 | | | | 6.00E−04 | | 7.60E−03 | | | 2B | X |
| Dibenz[*a*,*h*]anthracene | 53-70-3 | | | | 4.00E−04 | 4.00E−03 | | 4.00E−04 | | | 2A | X |
| Dibenzo[*b*,*def*]chrysene {dibenzo[*a*,*h*]pyrene} | 189-64-0 | | | | | | | | | | 2B | X |
| **Dibenzo[*def*,*p*]chrysene {dibenzo[a,*l*]pyrene}** [n] | 191-30-0 | | | | | | | | | | 2B | X |
| Fluoranthene | 206-44-0 | 4.11E−02 | | | 3.95E−02 | | | 3.95E−02 | | | | |
| Fluorene | 86-73-7 | 1.91E−01 | | | 8.84E−02 | | | 8.84E−02 | | | | |
| Indeno[1,2,3-*cd*]pyrene | 193-39-5 | 2.63E−03 | | | 3.50E−03 | | | 3.50E−03 | | | 2B | X |
| Naphthalene | 91-20-3 | 2.76E−01 | | | 3.42E−01 | | | 3.42E−01 | | | | X |
| Naphthalene, 1-methyl- | 90-12-0 | | | | 3.57E−01 | | | 3.57E−01 | | | | |
| Naphthalene, 2-methyl- | 91-57-6 | | | | 3.40E−01 | | | 3.40E−01 | | | | |
| Naphtho[1,2,3,4-*def*]chrysene {dibenzo[*a*,*e*]pyrene} | 192-65-4 | | | | | | | | | | 2B | X |
| Phenanthrene | 85-01-8 | 1.19E−01 | | | 9.20E−02 | | | 9.20E−02 | | | | X |
| Pyrene | 129-00-0 | 3.54E−02 | | | 2.45E−02 | | | 2.45E−02 | | | | X |

**Table 1 (*cont.*)**

| Agent | CAS no. | INBIFO control [a] µg/cig | RJR 1R4F [b,c,d] µg/cig | Omni 1R4F [e] µg/cig | Rickert 1R4F [f] µg/cig | Hoffmann *et al.* [g] max. µg/cig | Misc. source µg/cig | Comparison cig value µg/cig | US CPSC test [h] | Canadian gov't test [i] | IARC class [j,k,l] | EPCRA sect. 313 [m] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Aza-arenes* | | | | | | | | | | | | |
| **7*H*-Dibenzo[*c,g*]carbazole** [o] | 194-59-2 | | | | | 7.00E−04 | | 7.00E−04 | | | 2B | X |
| Anabasine | 494-52-0 | | | | | 1.20E+01 | | 1.20E+01 | | | | |
| Carbazole | 86-74-8 | | | | | | [p] | 1.00E+00 | | | | |
| Carbazole, 9-methyl- | 1484-12-4 | | | | | | [q] | 1.00E−01 | | | | |
| **Dibenz[*a,h*]acridine** [o] | 226-36-8 | | | | | 1.00E−04 | | 1.00E−04 | | | 2B | X |
| **Dibenz[*a,j*]acridine** [o] | 224-42-0 | 2.72E−03 | | | | 1.00E−02 | | 2.72E−03 | | | 2B | X |
| Indole | 120-72-9 | | | | | 1.50E+01 | | 1.50E+01 | | | | |
| Indole, 1-methyl- | 603-76-9 | | | | | | [q] | 8.00E−01 | | | | |
| Nicotine | 54-11-5 | 7.40E+02 | 7.90E+02 | 7.80E+02 | 7.86E+02 | 3.00E+03 | | 7.90E+02 | X | X | | X |
| Pyridine | 110-86-1 | | 2.10E+00 | 4.53E+00 | 7.56E+00 | 4.00E+01 | | 7.56E+00 | | X | | X |
| Pyridine, 3-ethenyl- | 1121-55-7 | | | 1.46E+00 | | 3.00E+01 | | 1.46E+00 | | | | |
| Pyridine, 2-methyl- | 109-06-8 | | | | | | [r] | 3.50E+00 | | | | X |
| Pyridine, 3-methyl- | 108-99-6 | | | | | | [r] | 4.80E+00 | | | | |
| Pyridine, 4-methyl- | 108-89-4 | | | | | | [r] | 6.00E−01 | | | | |
| Quinoline | 91-22-5 | | 2.30E−01 | 2.66E−01 | 3.15E−01 | 2.00E+00 | | 3.15E−01 | | X | | X |
| *Aromatic amines* | | | | | | | | | | | | |
| Aniline | 62-53-3 | | | | | 6.55E−01 | | 6.55E−01 | | | 3 | X |
| Aniline, 2,6-dimethyl- | 87-62-7 | | | | | 5.00E−02 | | 5.00E−02 | | | 2B | X |
| Aniline, 2-methyl- | 95-53-4 | | | | | 2.00E−01 | | 2.00E−01 | | | 2B | X |
| Biphenyl, 3-amino- | 2243-47-2 | | | | 3.16E−03 | | | 3.16E−03 | | X | | |
| Biphenyl, 4-amino- | 92-67-1 | | 4.00E−03 | | 2.15E−03 | 5.60E−03 | | 4.00E−03 | | X | 1 | X |
| Naphthalene, 1-amino- | 134-32-7 | | | | 1.68E−02 | | | 1.68E−02 | | X | | X |
| Naphthalene, 2-amino- | 91-59-8 | | 1.09E−02 | | 1.11E−02 | 2.20E−02 | | 1.11E−02 | | X | 1 | X |
| *N-Heterocyclic amines* | | | | | | | | | | | | |
| A$\alpha$C | 26148-68-5 | | | | | 2.60E−01 | | 2.60E−01 | | | 2B | |
| Glu-P-1 | 67730-11-4 | | | | | 8.90E−04 | | 8.90E−04 | | | 2B | |
| Glu-P-2 | 67730-10-3 | | | | | 8.80E−04 | | 8.80E−04 | | | 2B | |
| IQ | 76180-96-6 | | | | | 3.00E−04 | | 3.00E−04 | | | 2A | |
| MeA$\alpha$C | 68006-83-7 | | | | | 3.70E−02 | | 3.70E−02 | | | 2B | |
| MeIQ | 77094-11-2 | | | | | | [l] | 7.50E−04 | | | 2B | |
| PhIP | 105650-23-5 | | | | | 2.30E−02 | | 2.30E−02 | | | 2B | |
| Trp-P-1 | 62450-06-0 | | | | | 5.00E−04 | | 5.00E−04 | | | 2B | |
| Trp-P-2 | 62450-07-1 | | | | | 1.10E−03 | | 1.10E−03 | | | 2B | |
| *N-Nitrosamines* | | | | | | | | | | | | |
| 4-(*N*-Methylnitrosamino)-1-(3-pyridinyl)-1-butanone | 64091-91-4 | 1.38E−01 | 9.70E−02 | 8.70E−02 | 8.74E−02 | 7.70E−01 | | 9.70E−02 | X | X | 2B | |
| *N'*-Nitrosoanabasine | 37620-20-5 | 1.86E−02 | | 1.92E−02 | 2.31E−02 | | | 2.31E−02 | | X | 3 | |

**Table 2 (*cont.*)**

| Agent | CAS no. | INBIFO control [a] µg/cig | RJR 1R4F [b,c,d] µg/cig | Omni 1R4F [e] µg/cig | Rickert 1R4F [f] µg/cig | Hoffmann *et al.* [g] max. µg/cig | Misc. source µg/cig | Comparison cig value µg/cig | US CPSC test [h] | Canadian gov't test [i] | IARC class [j,k,l] | EPCRA sect. 313 [m] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *N-Nitrosamines (cont.)* | | | | | | | | | | | | |
| *N'*-Nitrosoanatabine | 71267-22-6 | | | | 1.22E−01 | | | 1.22E−01 | | X | 3 | |
| *N'*-Nitrosonornicotine | 16543-55-8 | 1.24E−01 | 1.15E−01 | 1.14E−01 | 1.07E−01 | 3.70E+00 | | 1.15E−01 | X | X | 2B | X |
| ***N-N*itrosodiethanolamine** [s] | 1116-54-7 | 4.30E−03 | | | | 6.80E−02 | | 4.30E−03 | | | 2B | |
| *N-N*itrosodiethylamine | 55-18-5 | | | | | 2.80E−03 | | 2.80E−03 | X | | 2A | X |
| *N*-Nitrosodimethylamine | 62-75-9 | 4.40E−03 | | | 1.91E−02 | 1.80E−01 | | 1.91E−02 | X | X | 2A | X |
| *N*-Nitrosodi-*n*-butylamine | 924-16-3 | | | | | 3.00E−02 | | 3.00E−02 | | | 2B | X |
| *N*-Nitrosodi-*n*-propylamine | 621-64-7 | | | | | 1.00E−03 | | 1.00E−03 | | | 2B | X |
| *N*-Nitrosoethylmethylamine | 10595-95-6 | | | | | 1.30E−02 | | 1.30E−02 | | | 2B | |
| *N-N*itroso-*n*-butylmethylamine | 7068-83-9 | | | | | | | | | | | X |
| *N*-Nitrosopiperidine | 100-75-4 | | | | | | [l] | 2.31E−01 | | | 2B | X |
| *N*-Nitrosopyrrolidine | 930-55-2 | 1.25E−02 | 1.40E−02 | | 5.15E−03 | 1.10E−01 | | 1.40E−02 | X | X | 2B | |
| *Aldehydes* | | | | | | | | | | | | |
| Acetaldehyde | 75-07-0 | 5.18E+02 | 6.40E+02 | 6.25E+02 | 5.54E+02 | 1.40E+03 | | 6.40E+02 | X | X | 2B | X |
| Acrolein | 107-02-8 | 4.63E+01 | 6.50E+01 | 6.24E+01 | 4.58E+01 | 1.40E+02 | | 6.50E+01 | X | X | 3 | X |
| Butyraldehyde | 123-72-8 | | | 3.94E+01 | 3.21E+01 | | | 3.94E+01 | | X | | X |
| Crotonaldehyde | 123-73-9 | | | 2.18E+01 | 1.29E+01 | | | 2.18E+01 | | X | 3 | X |
| Formaldehyde | 50-00-0 | 1.65E+01 | 8.50E+00 | 2.31E+01 | 1.98E+01 | 1.00E+02 | | 2.31E+01 | | X | 2A | X |
| Furfural | 98-01-1 | | 1.40E+00 | | | | | 1.40E+00 | | | | |
| Propionaldehyde | 123-38-6 | | | | 4.94E+01 | | | 4.94E+01 | X | X | | X |
| *Acids* | | | | | | | | | | | | |
| Acetic acid | 64-19-7 | | | | | | [p] | 1.00E+03 | | | | |
| Formic acid | 64-18-6 | | | | | 6.00E+02 | | 6.00E+02 | | | | X |
| Propionic acid | 79-09-4 | | | | | 3.00E+02 | | 3.00E+02 | | | | |
| *Ketones* | | | | | | | | | | | | |
| 2,3-Butanedione | 57-71-6 | | | | | | [t] | 1.65E+02 | | | | |
| 2-Butanone | 78-93-3 | | | 9.00E+01 | 6.34E+01 | | | 9.00E+01 | | X | | X |
| Acetone | 67-64-1 | | 2.84E+02 | 2.75E+02 | 2.91E+02 | | | 2.91E+02 | | X | | |
| *Phenols* | | | | | | | | | | | | |
| Caffeic acid | 331-39-5 | | | | | 3.00E+00 | | 3.00E+00 | | | 2B | |
| Catechol | 120-80-9 | 5.38E+01 | 4.53E+01 | 3.90E+01 | 3.99E+01 | 3.60E+02 | | 4.53E+01 | X | X | 2B | X |

**Table 2 (*cont.*)**

| Agent | CAS no. | INBIFO control [a] µg/cig | RJR 1R4F [b,c,d] µg/cig | Omni 1R4F [e] µg/cig | Rickert 1R4F [f] µg/cig | Hoffmann *et al.* [g] max. µg/cig | Misc. source µg/cig | Comparison cig value µg/cig | US CPSC test [h] | Canadian gov't test [i] | IARC class [j,k,l] | EPCRA sect. 313[m] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Phenols (cont.)* | | | | | | | | | | | | |
| Eugenol, methyl- | 93-15-2 | | | | | 2.00E−02 | | 2.00E−02 | | | | |
| Hydroquinone | 123-31-9 | 4.33E+01 | 4.29E+01 | 3.44E+01 | 4.06E+01 | | | 4.29E+01 | | X | | X |
| Phenol | 108-95-2 | 1.18E+01 | 8.90E+00 | 1.05E+01 | 9.79E+00 | 1.60E+02 | | 1.05E+01 | X | X | | X |
| Phenol, 2-methyl- | 95-48-7 | 3.31E+00 | | 3.10E+00 | 3.33E+00 | | | 3.33E+00 | | X | | X |
| Phenol, 3-methyl- | 108-39-4 | 2.55E+00 | 2.17E+00 [u] | 2.26E+00 [u] | 2.25E+00 [u] | | | 2.26E+00 | | X | | X |
| Phenol, 4-methyl- | 106-44-5 | 6.36E+00 | 5.43E+00 [u] | 5.64E+00 [u] | 5.62E+00 [u] | | | 5.64E+00 | | X | | X |
| Resorcinol | 108-46-3 | | | 6.40E−01 | 5.44E−01 | | | 6.40E−01 | | X | | X |
| *Volatile hydrocarbons* | | | | | | | | | | | | |
| Benzene | 71-43-2 | 3.98E+01 | 4.42E+01 | 4.79E+01 | 3.90E+01 | 7.00E+01 | | 4.79E+01 | X | X | 1 | X |
| 1,3-Butadiene | 106-99-0 | 4.27E+01 | 3.65E+01 | 4.10E+01 | 3.71E+01 | 7.50E+01 | | 4.10E+01 | X | X | 2A | X |
| *D*-Limonene | 5989-27-5 | | | | | | [v] | 6.00E+01 | | | | |
| Isoprene | 78-79-5 | 3.19E+02 | 3.66E+02 | 4.47E+02 | 3.49E+02 | 1.00E+03 | | 4.47E+02 | X | X | 2B | |
| Styrene {benzene, ethenyl-} | 100-42-4 | | 2.10E+00 | 7.60E+00 | 6.75E+00 | 1.00E+01 | | 7.60E+00 | | X | 2B | X |
| Toluene | 108-88-3 | 6.72E+01 | 7.33E+01 | 9.04E+01 | 6.35E+01 | | | 9.04E+01 | X | X | | X |
| *Polychlorinated heterocycles* | | | | | | | | | | | | |
| Polychlorodibenzo-*p*-dioxins | | | | | | | [w] | 7.50E−06 | | | 1 | X |
| Polychlorodibenzofurans | | | | | | | [w] | 2.98E−06 | | | 3 | X |
| *Organic nitro compounds* | | | | | | | | | | | | |
| Nitrobenzene | 98-95-3 | | | | | 2.50E+01 | | 2.50E+01 | | | 2B | X |
| Nitromethane | 75-52-5 | | | | | 6.00E−01 | | 6.00E−01 | | | 2B | |
| Propane, 2-nitro- | 79-46-9 | | | | | 2.20E+00 | | 2.20E+00 | | | 2B | X |
| *Miscellaneous organic compounds* | | | | | | | | | | | | |
| Acetamide | 60-35-5 | | 3.97E+00 | | | 5.60E+01 | | 3.97E+00 | | | 2B | X |
| Acrylamide | 79-06-1 | | 2.20E+00 | | | | | 2.20E+00 | | | 2A | X |
| Acetonitrile | 75-05-8 | | | | | | [x] | 1.00E+02 | | | | X |
| Acrylonitrile | 107-13-1 | 9.05E+00 | 1.39E+01 | 1.09E+01 | 8.58E+00 | 1.50E+01 | | 1.39E+01 | | X | 2B | X |
| Benzo[*b*]furan | 271-89-6 | | | | | | | | | | 2B | |
| Carbon disulfide | 75-15-0 | | | | | | [y] | 2.00E+00 | | | | X |
| Carbon monoxide | 630-08-0 | 1.00E+04 | 1.13E+04 | 1.18E+04 | 1.24E+04 | 2.30E+04 | | 1.24E+04 | X | X | | |
| Carbonyl sulfide | 463-58-1 | | | | | | [y] | 3.50E+01 | | | | X |
| Cyanogen | 460-19-5 | | | | | 1.10E+01 | | 1.10E+01 | | | | |
| **DDE** [z] | 72-55-9 | | | | | 3.70E−01 | | 3.70E−01 | | | 2B | |
| **DDT** [z] | 50-29-3 | | | | | 1.20E+00 | | 1.20E+00 | | | 2B | |
| Dimethylamine | 124-40-3 | | | | | | [aa] | 1.00E+00 | | | | X |

**Table 1 (*cont.*)**

| Agent | CAS no. | INBIFO control [a] µg/cig | RJR 1R4F [b,c,d] µg/cig | Omni 1R4F [e] µg/cig | Rickert 1R4F [f] µg/cig | Hoffmann *et al.* [g] max. µg/cig | Misc. source µg/cig | Comparison cig value µg/cig | US CPSC test [h] | Canadian gov't test [i] | IARC class [j,k,l] | EPCRA sect. 313 [m] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Miscellaneous organic compounds (cont.)* | | | | | | | | | | | | |
| Ethyl carbamate {urethane} | 51-79-6 | | | | | 3.80E+01 | | 3.80E+01 | | | 2B | X |
| Ethylene oxide | 75-21-8 | | | | | 7.00 E+00 | | 7.00 E+00 | | | 1 | X |
| Ethylenethiourea | 96-45-7 | | | | | | l | 2.70 E−02 | | | 2B | X |
| Furan | 110-00-9 | | | | | 3.00 E+01 | | 3.00 E+01 | | | 2B | |
| γ-Butyrolactone | 96-48-0 | | | | | | bb | 1.10 E+01 | | | | |
| Hydrazine, 1,1-dimethyl- | 57-14-7 | | | | | | | | | | 2B | X |
| Hydrogen cyanide | 74-90-8 | 8.08 E+01 | 1.65 E+02 | 1.03 E+02 | 1.20 E+02 | 5.00 E+02 | | 1.65 E+02 | X | X | | X |
| Hydrogen sulfide | 7783-06-4 | | | | | 9.00 E+01 | | 9.00 E+01 | | | | |
| **Nickel carbonyl** [cc] | 13463-39-3 | | | | | | | | | | | |
| Maleic hydrazide | 123-33-1 | | | | | 1.16 E+00 | | 1.16 E+00 | | | | |
| Methanol | 67-56-1 | | | | | | v | 1.80 E+02 | | | | X |
| Methyl formate | 107-31-3 | | | | | 3.00 E+01 | | 3.00 E+01 | | | | |
| Methyl isocyanate | 624-83-9 | | | | | 5.00 E+00 | | 5.00 E+00 | | | | X |
| Methylamine | 74-89-5 | | | | | 1.00 E+01 | | 1.00 E+01 | | | | |
| **Di(2-ethylhexyl) phthalate** | 117-81-7 | | | | | | v | 2.00 E+01 | | | | X |
| Propylene oxide | 75-56-9 | | | | | 1.00 E−01 | | 1.00 E−01 | | | 2B | X |
| Quinone | 106-51-4 | | | | | | | P | | | | X |
| Vinyl acetate | 108-05-4 | | | | | | l | 4.00 E+00 | | | 2B | X |
| Vinyl chloride | 75-01-4 | 3.00 E−02 | | | | 1.60 E−02 | | 3.00 E−02 | | | 1 | X |
| *Inorganic compounds* | | | | | | | | | | | | |
| Ammonia | 7664-41-7 | | 1.60 E+01 | | 1.30 E+01 | 1.30 E+02 | | 1.60 E+01 | | X | | X |
| Hydrazine | 302-01-2 | | | | | 4.30 E−02 | | 4.30 E−02 | | | 2B | X |
| Nitric oxide | 10102-43-9 | 2.63 E+02 | 2.58 E+02 | 2.38 E+02 | 2.76 E+02 | 6.00 E+02 | | 2.76 E+02 | X | X | | |
| Sulfur dioxide | 7446-09-5 | | | | | | aa | 4.20 E+00 | | | | |
| *Metals* | | | | | | | | | | | | |
| Arsenic | 7440-38-2 | 3.33 E−03 | | 5.80 E−03 | 4.59 E−03 | 1.20 E+02 | | 5.80 E−03 | | X | 1 | X |
| Beryllium | 7440-41-7 | | | | | 5.00 E−04 | | 5.00 E−04 | | | 1 | X |
| Cadmium | 7440-43-9 | 2.47 E−02 | | 6.42 E−02 | 6.73 E−02 | 3.50 E−01 | | 6.73 E−02 | | X | 1 | X |
| Chromium | 7440-47-3 | | | | 4.31 E−03 | | | 4.31 E−03 | | X | 1 | X |
| Chromium VI | 1333-82-0 | 1.32 E−03 | | | | 7.00 E−02 | | 1.32 E−03 | | | 1 | X |
| Cobalt | 7440-48-4 | | | 3.76 E−02 | | 2.00 E−04 | | 3.76 E−02 | | | 2B | X |
| Lead | 7439-92-1 | 1.01 E−02 | | 3.76 E−02 | 3.91 E−02 | 8.50 E−02 | | 3.91 E−02 | | X | 2B | X |
| Mercury | 7439-97-6 | | | | 5.96 E−03 | 4.00 E−03 | | 5.96 E−03 | | X | | X |
| Nickel | 7440-02-0 | 2.63 E−03 | | | 5.58 E−03 | 6.00 E−01 | | 5.58 E−03 | | X | 1 | X |

**Table 1 (*cont.*)**

| Agent | CAS no. | INBIFO control [a] µg/cig | RJR 1R4F [b,c,d] µg/cig | Omni 1R4F [e] µg/cig | Rickert 1R4F [f] µg/cig | Hoffmann *et al.* [g] max. µg/cig | Misc. source µg/cig | Comparison cig value µg/cig | US CPSC test [h] | Canadian gov't test [i] | IARC class [j,k,l] | EPCRA sect. 313 [m] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Metals* | | | | | | | | | | | | |
| Polonium-210 (pCi) | 7440-08-6 | | 1.60 E−02 | | | 1.00 E+00 | [dd] | 1.60 E−02 | | | 1 | |
| Selenium | 7782-49-2 | | | | 1.20 E−03 | | | 1.20 E−03 | | X | | X |

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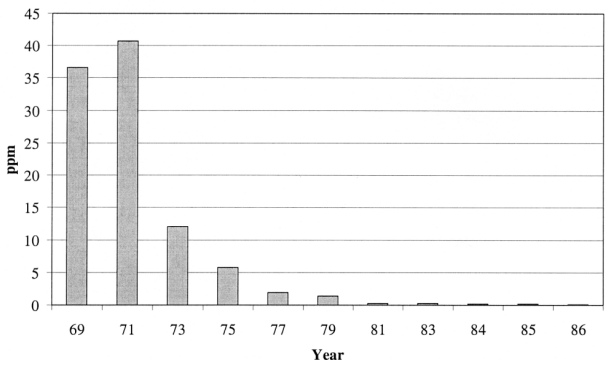



**Figure 1. Historical trend of TDE + DDT residues in US cigarettes (ppm)**

has been replaced by the potassium salt of maleic hydrazide. Analyses of tobacco grown under inhibitor-free conditions and the smoke generated from such tobacco failed to show the presence of NDELA (BRUNNEMANN and HOFFMANN, 40).

Despite the fact that in 1984 HOFFMANN *et al.* (41) predicted that NDELA in tobacco and its smoke would decrease due to the 1981 ban on the use of the diethanolamine salt of maleic hydrazide and their prediction has come true since 1981, HOFFMANN and his colleagues persist in listing NDELA as a significant tumorigen or biologically active component of cigarette MSS. As reviewed by RODGMAN (42), the diminution of levels of NDELA in tobacco should parallel the chronicled decrease in arsenic and DDT levels in tobacco after these materials were no longer used in tobacco agronomy. Between 1968 and 1974, the residual level of DDT in USA grown flue-cured tobacco decreased from a range of 28 to 52 μg/g in 1968 to 6 μg/g in 1970 to 0.23 μg/g in 1974 [USPHS (43), see p. 61; IARC (44)]. In the late 1960s, the transfer rate of DDT from cigarette tobacco to its MSS was reported as 5% by NESEMANN *et al.* (45) and as 12% by HOFFMANN *et al.* (46). With these percent transfers and a cigarette tobacco level of 0.23 μg/g, the MSS would contain either 11 or 28 ng/cig of DDT. In the 1979 Surgeon General's report it was noted that a significant reduction of the use of chlorinated hydrocarbon insecticides resulted in reduced residues on the tobacco (43). In a 1979 review, SHEETS and LEIDY (47) reported that the average DDT level in US flue-cured tobacco was 0.13 μg/g. Later, TSO (48) summarized most of these data. In 1991, SHEETS (49) summarized some unpublished data on DDT in US commercial cigarettes: The amounts of DDT (sum of the three isomers) in 19 commercial cigarette brands ranged from 0.11 to 0.28 μg/g, averaging 0.19 μg/g. The historical trend of DDT levels is shown graphically in Figure 1 (50).

Over time, similar decreases were reported for arsenic residues, usually considered as $As_2O_3$, in tobacco after arsenic use was removed from tobacco agronomy in 1952. Between 1917 and 1951 the arsenic level in tobacco rose from about 12 to 57 μg/g (51). By 1968 the arsenic level in tobacco had decreased from the 1951 value of more than 50 μg/g to a 1968 value of 0.5–1.0 μg/g, a value similar to that reported by GRIFFIN *et al.* (52). Some of these chronological data were summarized by the US Surgeon General in 1979 [see p. 59 in (43)] and IARC (44). In 1957, COGBILL and HOBBS (53) reported the transfer of arsenic from a cigarette containing 7.1 μg of arsenic to its MSS to be 3.5%. With the tobaccos analyzed for arsenic by GRIFFIN *et al.*, the arsenic content of the MSS would range from 0.018 to 0.035 μg/cig. In 1968, GUTHRIE (54) reported the arsenic transfer from cigarette tobacco to its MSS varied between 4% and 12%. In 1990, TSO (48) noted that for most tobaccos at that time the arsenic level was around 0.1 to 0.5 μg/g.

## 5 UNCORROBORATED SMOKE TOXICANTS

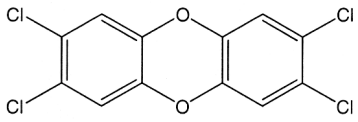
Among the toxicants listed in bold font in Table 1 are three aza-arenes, i.e., 7*H*-dibenzo[*c,g*]carbazole, dibenz[*a,h*]acridine, and dibenz[*a,j*]acridine. All three of these compounds were first reported in cigarette MSS by VAN DUUREN *et al.* (55) and subsequently CANDELI *et al.* [(56), see pp. 373–374, Table VIII-14 in (57)] reported unpublished data indicating that dibenz[*a,j*]acridine is a smoke toxicant. It has long been known that these three compounds are biologically active. However, as RODGMAN has documented (58), "Despite numerous attempts in Japan, Germany, and the USA between 1960 and 1992 to confirm the presence of these three aza-arenes in cigarette MSS and nicotine pyrolysates, the 1960 findings reported . . . have not been confirmed . . ." Additionally, since the Rodgman report, SASAKI and MOLDOVEANU (59) have attempted to resolve the controversy related to the presence of the two dibenzacridines in cigarette MSS. Even through the use of selected ion monitoring gas chromatography-mass spectrometry and dibenz[*a,j*]acridine-$d_{13}$ as an internal standard, SASAKI and MOLDOVEANU were unable to detect the presence of either dibenzacridine in smoke condensate.

Although the absence of any compound in cigarette smoke is impossible to prove, enough modern analytical studies have been performed to remove 7*H*-dibenzo[*c,g*]carbazole, dibenz[*a,h*]acridine, and dibenz[*a,j*]acridine from the list of smoke toxicants without further proof of their existence.

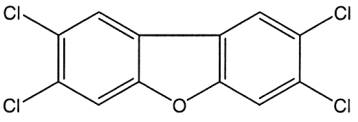
## 6 POLYCHLORODIBENZO-*p*-DIOXINS AND POLYCHLORODIBENZOFURANS

Among the smoke toxicants conspicuous in their absence from all toxicant lists except that of FOWLES and BATES (33) are the polychlorodibenzo-*p*-dioxins (PCDDs) and polychlorodibenzofurans (PCDFs). The presence of dioxins in cigarette smoke was first reported in 1980 by CRUMMETT (60). More recently, there are at least five publications reporting the presence of dioxins (PCDDs and PCDFs) in cigarette tobacco or its MSS. These include in chronological order of publication: MUZO and TAKIZAWA (61), BALL *et al.* (62), MATSUEDA *et al.* (63), LÖFROTH and ZEBÜHR (64), and Matsueda *et al.* (65). Example compounds are shown in Figure 2.

The smoke yield data of MUZO and TAKIZAWA (61) come from a single smoking puff that entirely consumes the cigarette and is clearly not appropriate for our quantitative risk assessment. The mainstream and sidestream data of LÖFROTH and ZEBÜHR (64) are derived from only one Swedish cigarette brand. The PCDD and PCDF data in the paper by




2,3,7,8-Tetrachlorodibenzo-*p*-dioxin




2,3,7,8-Tetrachlorodibenzofuran

**Figure 2. Example polychlorodibenzo-*p*-dioxin and -furan**

MATSUEDA *et al*. (65) are for the amount of these compounds contained in the cigarette tobacco, rather than the smoke. The smoke yield data for the BALL *et al*. (62) and MATSUEDA *et al*. (63) experiments are similar. Because the BALL *et al*. data were collected and analyzed by a well-validated method, and the laboratory where the analyses were performed, i.e., ERGO Forschungsgesellschaft mbH, Hamburg, has been accredited by the World Health Organization (WHO) for dioxin analysis, we have chosen those results for further analysis.

The analytical data of BALL *et al*. represent results from the ten top selling brands in Germany during the fourth quarter of 1989. The ERGO scientists chose to present individual data on each of the tested cigarettes. It should be noted that the most toxic isomer, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was not detected in any of the samples and additionally, not every isomer present was quantifiable in each product tested. For the purposes of this paper we have summarized the average cigarette data in Table 2.

As may be seen listed in Table 2, the total amount of PCDDs and PCDFs is 7.50 and 2.98 pg/cig, respectively. Incorporating all of the various isomers with their individual toxicities into a risk assessment is difficult. Therefore, toxicologists have determined the absolute toxicity of the most potent congener, i.e., TCDD and related the toxicity of all other congeners to the most potent one. The total toxicity potential of a mixture of PCDDs and PCDFs is expressed as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity equivalents (TEQs). However, among US EPA and WHO scientists, there is disagreement over how to calculate the TEQs. Thus, Table 2 shows mean values of TCDD TEQs of 1.258 and 0.0975 pg/cig, respectively. For all further calculations, we will use the US EPA value because it is greater and any risk based upon its value may be biased high.

**Table 2. Polychlorodibenzo-*p*-dioxins and -furans in cigarette mainstream smoke** [a] (results in pg/cig [b])

| Compound | Average | US EPA-TEQ [c,d] | WHO-TEQ [e] |
|---|---|---|---|
| 2,3,7,8-Tetra-CDD [f] | | | |
| Sum tetra-CDD | 0.51 | 0.00507 | |
| 1,2,3,7,8-Penta-CDD | | | |
| Sum penta-CDD | 0.15 | 0.0145 | |
| 1,2,3,4,7,8-Hexa-CDD | 0.08 | | 0.00771 |
| 1,2,3,6,7,8-Hexa-CDD | 0.06 | | 0.00600 |
| 1,2,3,7,8,9-Hexa-CDD | 0.04 | | 0.00414 |
| Sum hexa-CDD | 0.53 | 0.0528 | |
| 1,2,3,4,6,7,9-Hepta-CDD | 1.61 | | |
| 1,2,3,4,6,7,8-Hepta-CDD | 1.29 | | 0.0129 |
| Sum hepta-CDD | 2.90 | 0.0290 | |
| Octa-CDD | 3.42 | 0.000342 | 0.000342 |
| 2,3,7,8-Tetra-CDF [g] | 0.19 | | 0.0187 |
| Sum tetra-CDF | 1.41 | 0.705 | |
| 1,2,3,7,8/1,2,3,4,8-Penta-CDF | 0.13 | | 0.00630 |
| 2,3,4,7,8-Penta-CDF | 0.04 | | 0.0206 |
| Sum penta-CDF | 0.83 | 0.414 | |
| 1,2,3,4,7,8/1,2,3,4,7,9-Hexa-CDF | 0.03 | | 0.00300 |
| 1,2,3,6,7,8-Hexa-CDF | 0.05 | | 0.00467 |
| 1,2,3,7,8,9-Hexa-CDF | 0.07 | | 0.00650 |
| 2,3,4,6,7,8-Hexa-CDF | 0.05 | | 0.00471 |
| Sum hexa-CDF | 0.35 | 0.0350 | |
| 1,2,3,4,6,7,8-Hepta-CDF | 0.16 | | 0.00157 |
| 1,2,3,4,6,7,9-Hepta-CDF | 0.04 | | 0.000360 |
| Sum hepta-CDF | 0.27 | 0.00267 | |
| Hecta-CDF | 0.15 | 0.0000154 | 0.0000154 |
| Sum PCDD | 7.50 | | |
| Sum PCDF | 2.98 | | |
| Sum PCDD/PCDF | 10.5 | | |
| TEQ (2,3,7,8-tetra-CDD units) | | 1.258 | 0.0975 |

[a] Ball, M., O. Päpke, and A. Lis: Polychlordibenzodioxine und Polychlordibenzofurane in Cigarettenrauch; Beitr. Tabakforsch. Int. 14 (1990) 393–402.

[b] Cigarettes analyzed were the top ten sellers in the German market during the fourth quarter of 1989 and the reported value is the mean of the analytical results. In most cases, the mean represents values from all ten cigarettes; however, in some cases the analyte was not detected, not analyzable, etc. and the mean of all reported values was used.

[c] Environmental Protection Agency: Health assessment for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds; Draft Document EPA/600/P-00/001Ae (May 2000), Chapter 9, Table 9-1, p. 9-35.

[d] TEQ is the amount of any polychlorodibenzo-*p*-dioxin or polychlorodibenzofuran expressed as toxic equivalent amounts of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

[e] *ibid.* reference c, Table 9-2, p. 9–36.

[f] CDD is an abbreviation for chlorodibenzo-*p*-dioxin.

[g] CDF is an abbreviation for chlorodibenzofuran.

## 7. QUANTITATIVE RISK ASSESSMENTS

> When you come to a fork in the road, take it.
>
> — *Yogi Berra* (66)

Heretofore, when tobacco scientists prepared a list of known cigarette MSS toxicants, they have for the most part listed the substances by name, amounts reported in smoke, whether or not it is believed to be a carcinogen, promoter, etc., and various other data. However, this type of listing appears to be of little use to researchers attempting to produce a "less hazardous" cigarette or to aid chemical analysts in prioritization of smoke components that require good analytical methods. Although the prohibitions against conducting quantitative risk assessments are legendary (9,

67–69) especially when it involves extrapolation of animal data to humans, it appears that we have reached the proverbial "fork in the road" mentioned in the quotation from Yogi Berra[1].

## 8 COMPARISON OF WORKPLACE EXPOSURE LIMITS WITH SMOKING EXPOSURE

The next part of our review will read like a US government report because of our repeated use of so many abbreviations/acronyms to shorten the discussion.

There have been at least three comparisons of workplace exposure limits with smoking exposure. These include analyses by toxicologists at RJRT (30), VORHEES *et al*. (31), and RICKERT and KAISERMAN (25). Both the RJRT toxicologists and VORHEES *et al*. used American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit values (TLVs) based upon eight-hour time weighted average exposures while RICKERT and KAISERMAN used 15-minute short-term ACGIH exposure limits (STELs) as their safe exposure value. Although it may be argued that the ACGIH STELs are the appropriate standard for comparison, they have the problem of being few in number. To have a comparison among a significant number of smoke toxicants several assumptions must be made, e.g., ACGIH threshold limit values (TLVs) and OSHA or NIOSH permissible exposure levels (PELs) can be substituted for STELs, ACGIH STELs can be compared with TLVs or PELs, and carcinogens that do not have STELs can be assigned the smallest tabled ACGIH STEL value, i.e., 0.0005 mg/m$^3$ that was developed for beryllium. Additionally, ACGIH STELs and TLVs are not enforceable standards and ACGIH has recently been sued for *de facto* illegally promulgating standards (70).

The RJRT analysis does not address any specific toxicant, but finds that "with very few exceptions, smoke constituents that occur in smoke at levels of 0.5 µg/cig or less present little or no potential for concern". Meanwhile, although VORHEES *et al*. state that, "The use of TLVs to derive ADIs [note added, acceptable daily intake] is inappropriate because TLVs were developed by the ACGIH specifically for workplace exposures rather than the broad population exposures expected with cigarette smoking", the VORHEES *et al*. objection to the use of TLVs is incomprehensible because the working population and smoking population are almost indistinguishable. Nevertheless, VORHEES *et al*. proceed to rank smoke toxicants based upon cigarette yields, number of cigarettes smoked per day, a 10-m$^3$/day inhalation rate for an eight-hour work period, and a body weight of 70 kg.

In the analysis that follows, we have simplified the smoke toxicant ranking process. Instead of ACGIH TLVs, the legally enforceable workplace permissible exposure level, i.e., $TWA_8$ in mg/m$^3$ approved by the US OSHA, is compared with the average daily concentration (ADC) that a pack-a-day smoker breathing 10 m$^3$ of air would obtain during a smoking workday. The calculation is as follows:

$$\text{ADC, mg/m}^3 = \frac{\text{per cig yield, mg} \times \text{20 cig per day}}{\text{10 m}^3\text{ breathing volume}}$$

$$\%\ \text{OSHA TWA}_8 = \frac{\text{ADC, mg/m}^3 \times 100}{\text{OSHA TWA}_8}$$

From the results shown in Table 3, it is perfectly acceptable for an employee to work in an area where the % of OSHA $TWA_8$ is less than 100% . Of course this assumes that none of the listed compounds has a short-term exposure limit (STEL) that would be exceeded by smoking one cigarette. For the agents listed, none of the STELs is exceeded by smoking one cigarette.

Nicotine is the only smoke component that exceeds the $TWA_8$ permissible concentration. However, acrolein, carbon monoxide, methyl isocyanate, and formic acid concentrations are reasonably high.

Typically, OSHA does not deal with occupational exposure to known carcinogens such as B*a*P, 2-aminonaphthalene, etc. other than to note that exposures to these materials should be eliminated either by engineering controls or respiratory protection. However, you will see listed in Table 3, a number of compounds that are considered to be carcinogens, e.g., ethylene oxide, acrylamide, acrylonitrile, benzene. These are exceptions to the general rule.

## 9 RANKING OF SMOKE TOXICANTS BY CARCINOGENIC POTENCY DATABASE VALUES

There is a general chemophobia among the US population. This fear of chemicals is fueled by presentations or publications in the mass media by advocacy groups who lay the blame for many adverse human health effects based upon the existence of trace synthetic, i.e., manmade, chemicals in our environment. Recent examples of this fear mongering may be found in a May 10$^{th}$, 2002, Public Broadcasting System-aired television special (71) titled "Kids and Chemicals", which poses the question, "Are everyday chemicals harming our kids?" As reported in the June 17$^{th}$, 2002 Chemical and Engineering News editorial (72):

> The gist of this one-hour special is that children are unwittingly and constantly exposed to a soup of toxic chemicals–from pesticides to air pollution to lead in paint. Some of these chemicals are known to cause cancer or other health effects in animals, but many chemicals have not been tested for their impact on children.

Additionally, the Rockefeller Family Fund has published a series of full-page advertisements prepared by the Mount Sinai Center for Children's Health & the Environment (CCHE) (73) during June 2002 in the *New York Times* and other newspapers.

Beginning in the late 1980s, Bruce Ames, inventor of the *Salmonella* mutagenicity assay, and several of his colleagues at the University of California at Berkeley began to take a look at the data implicating human health effects from exposure to synthetic chemicals and comparing these chemical exposures with those from naturally-occurring sources. To accomplish this task, Ames and his co-workers developed the Carcinogenic Potency Database (CPDB) (74). A recent review by GOLD *et al.* (75) summarizes development, analysis, and conclusions reached through the use of the CPDB. Among key points cited in the review are the following:

[1] Yogi Berra was a baseball player for the New York Yankees during the 1940–50s who is famous in the US for his predilection for quotable quotes, e.g., "It's deja vu all over again".

**Table 3. Comparison of pack-a-day-smoker concentration with OSHA permissible 8-h time weighted average concentration [a]**. Agents in bold have been included in previous lists of MSS toxicants, but no longer appear to be relevant. See footnotes of Table 1 for details.

| Agent | CAS no. | Comp. cig value mg/cig | OSHA $TWA_8$ $mg/m^3$ | ADC for 20 cig per day [b] $mg/m^3$ | % of OSHA [c] $TWA_8$ |
|---|---|---|---|---|---|
| Nicotine | 54-11-5 | 7.90 E−01 | 5.00 E−01 | 1.58 E+00 | 316 |
| Acrolein | 107-02-8 | 6.50 E−02 | 2.50 E−01 | 1.30 E−01 | 52.0 |
| Carbon monoxide | 630-08-0 | 1.24 E+01 | 5.50 E+01 | 2.48 E+01 | 45.1 |
| Methyl isocyanate | 624-83-9 | 5.00 E−03 | 5.00 E−02 | 1.00 E−02 | 20.0 |
| Formic acid | 64-18-6 | 6.00 E−01 | 9.00 E+00 | 1.20 E+00 | 13.3 |
| Acetic acid | 64-19-7 | 1.00 E+00 | 2.50 E+01 | 2.00 E+00 | 8.00 |
| Formaldehyde | 50-00-0 | 2.31 E−02 | 9.22 E−01 | 4.62 E−02 | 5.01 |
| Hydroquinone | 123-31-9 | 4.29 E−02 | 2.00 E+00 | 8.58 E−02 | 4.29 |
| 1,3-Butadiene | 106-99-0 | 4.10 E−02 | 2.21 E+00 | 8.20 E−02 | 3.71 |
| Benzene | 71-43-2 | 4.79 E−02 | 3.19 E+00 | 9.58 E−02 | 3.00 |
| Hydrogen cyanide | 74-90-8 | 1.65 E−01 | 1.10 E+01 | 3.30 E−01 | 3.00 |
| Cadmium | 7440-43-9 | 6.73 E−05 | 5.00 E−03 | 1.35 E−04 | 2.69 |
| Propionic acid [d] | 79-09-4 | 3.00 E−01 | 3.00 E+01 | 6.00 E−01 | 2.00 |
| Nitric oxide | 10102-43-9 | 2.76 E−01 | 3.00 E+01 | 5.52 E−01 | 1.84 |
| Acrylamide | 79-06-1 | 2.20 E−03 | 3.00 E−01 | 4.40 E−03 | 1.47 |
| Nitrobenzene | 98-95-3 | 2.50 E−02 | 5.00 E+00 | 5.00 E−02 | 1.00 |
| **Di(2-ethylhexyl) phthalate** | 117-81-7 | 2.00 E−02 | 5.00 E+00 | 4.00 E−02 | 0.800 |
| Ethylene oxide | 75-21-8 | 7.00 E−03 | 1.80 E+00 | 1.40 E−02 | 0.778 |
| Crotonaldehyde | 123-73-9 | 2.18 E−02 | 6.00 E+00 | 4.36 E−02 | 0.727 |
| Hydrogen sulfide | 7783-06-4 | 9.00 E−02 | 2.80 E+01 | 1.80 E−01 | 0.643 |
| Acrylonitrile | 107-13-1 | 1.37 E−02 | 4.34 E+00 | 2.74 E−02 | 0.631 |
| Catechol [d] | 120-80-9 | 4.53 E−02 | 2.00 E+01 | 9.06 E−02 | 0.453 |
| Acetaldehyde | 75-07-0 | 6.40 E−01 | 3.60 E+02 | 1.28 E+00 | 0.356 |
| **DDT** | 50-29-3 | 1.20 E−03 | 1.00 E+00 | 2.40 E−03 | 0.240 |
| Acetonitrile | 75-05-8 | 1.00 E−01 | 7.00 E+01 | 2.00 E−01 | 0.286 |
| Methylamine | 74-89-5 | 1.00 E−02 | 1.20 E+01 | 2.00 E−02 | 0.167 |
| Lead | 7439-92-1 | 3.91 E−05 | 5.00 E−02 | 7.82 E−05 | 0.156 |
| Methanol | 67-56-1 | 1.80 E−01 | 2.60 E+02 | 3.60 E−01 | 0.138 |
| Arsenic | 7440-38-2 | 5.80 E−06 | 1.00 E−02 | 1.16 E−05 | 0.116 |
| Phenol, 4-methyl- | 106-44-5 | 5.64 E−03 | 1.00 E+01 | 1.13 E−02 | 0.113 |
| Phenol | 108-95-2 | 1.05 E−02 | 1.90 E+01 | 2.10 E−02 | 0.111 |
| Cyanogen | 460-19-5 | 1.10 E−02 | 2.00 E+01 | 2.20 E−02 | 0.110 |
| Pyridine | 110-86-1 | 7.56 E−03 | 1.50 E+01 | 1.51 E−02 | 0.101 |
| Ammonia | 7664-41-7 | 1.60 E−02 | 3.50 E+01 | 3.20 E−02 | 0.0914 |
| Cobalt | 7440-48-4 | 3.76 E−05 | 1.00 E−01 | 7.52 E−05 | 0.0752 |
| Sulfur dioxide | 7446-09-5 | 4.20 E−03 | 1.30 E+01 | 8.40 E−03 | 0.0646 |
| Beryllium | 7440-41-7 | 5.00 E−07 | 2.00 E−03 | 1.00 E−06 | 0.0500 |
| Phenol, 3-methyl- | 108-39-4 | 2.26 E−03 | 1.00 E+01 | 4.52 E−03 | 0.0452 |
| 2-Butanone | 78-93-3 | 9.00 E−02 | 5.90 E+02 | 1.80 E−01 | 0.0305 |
| Phenol, 2-methyl- | 95-48-7 | 3.33 E−03 | 2.20 E+01 | 6.66 E−03 | 0.0303 |
| Acetone | 67-64-1 | 2.91 E−01 | 2.40 E+03 | 5.82 E−01 | 0.0242 |
| Methyl formate | 107-31-3 | 3.00 E−02 | 2.50 E+02 | 6.00 E−02 | 0.0240 |
| Toluene | 108-88-3 | 9.04 E−02 | 7.54 E+02 | 1.81 E−01 | 0.0234 |
| Furfural | 98-01-1 | 1.40 E−03 | 2.00 E+01 | 2.80 E−03 | 0.0140 |
| Mercury | 7439-97-6 | 5.96 E−06 | 1.00 E−01 | 1.19 E−05 | 0.0119 |
| Dimethylamine | 124-40-3 | 1.00 E−03 | 1.80 E+01 | 2.00 E−03 | 0.0111 |
| Aniline | 62-53-3 | 6.55 E−04 | 1.90 E+01 | 1.31 E−03 | 0.00690 |
| Hydrazine | 302-01-2 | 4.30 E−05 | 1.30 E+00 | 8.60 E−05 | 0.00662 |
| Carbon disulfide | 75-15-0 | 2.00 E−03 | 6.22 E+01 | 4.00 E−03 | 0.00643 |
| Propane, 2-nitro- | 79-46-9 | 2.20 E−03 | 9.00 E+01 | 4.40 E−03 | 0.00489 |
| Styrene {benzene, ethenyl-} | 100-42-4 | 7.60 E−03 | 4.26 E+02 | 1.52 E−02 | 0.00357 |
| Resorcinol [d] | 108-46-3 | 6.40 E−04 | 4.50 E+01 | 1.28 E−03 | 0.00284 |
| Chromium VI | 1333-82-0 | 1.32 E−06 | 1.00 E−01 | 2.64 E−06 | 0.00264 |
| Vinyl chloride | 75-01-4 | 3.00 E−05 | 2.56 E+00 | 6.00 E−05 | 0.00234 |
| Aniline, 2-methyl- | 95-53-4 | 2.00 E−04 | 2.20 E+01 | 4.00 E−04 | 0.00182 |
| Naphthalene | 91-20-3 | 3.42 E−04 | 5.00 E+01 | 6.84 E−04 | 0.00137 |
| Selenium | 7782-49-2 | 1.20 E−06 | 2.00 E−01 | 2.40 E−06 | 0.00120 |
| Nickel | 7440-02-0 | 5.58 E−06 | 1.00 E+00 | 1.12 E−05 | 0.00112 |
| Chromium | 7440-47-3 | 4.31 E−06 | 1.00 E+00 | 8.62 E−06 | 0.000862 |

**Table 3 (*cont.*)**

| Agent | CAS no. | Comp. cig value mg/cig | OSHA $TWA_8$ $mg/m^3$ | ADC for 20 cig per day [b] $mg/m^3$ | % of OSHA [c] $TWA_8$ |
|---|---|---|---|---|---|
| Nitromethane | 75-52-5 | 6.00 E−04 | 2.50 E+02 | 1.20 E−03 | 0.000480 |
| Aniline, 2,6-dimethyl- | 87-62-7 | 5.00 E−05 | 2.50 E+01 | 1.00 E−04 | 0.000400 |
| Propylene oxide | 75-56-9 | 1.00 E−04 | 2.40 E+02 | 2.00 E−04 | 0.000083 |

[a] US Department of Health and Human Services: NIOSH pocket guide to chemical hazards (stand-alone HTML version); DHHS (NIOSH) Publication No. 2001-145, August 2001, www.cdc.gov/niosh/npg/npg.html.
[b] The average daily concentration (ADC) in $mg/m^3$ is computed by assuming a breathing volume of 10 $m^3$ during the smoking day and smoking 20 cigarettes during that period of time, i.e., ADC ($mg/m^3$) = comparison cig value (mg/cig) × 20 cig/day ÷ 10 $m^3$ inhaled volume.
[c] % of OSHA $TWA_8$ = ADC ($mg/m^3$) × 100 ÷ $TWA_8$ ($mg/m^3$).
[d] The NIOSH $TWA_8$ is used because none is established by OSHA.

- Half the chemicals tested in rodent assays are found to be carcinogens; this rate holds whether the chemical is manmade or naturally occurring.
- Among chemicals to which humans are exposed approximately 99.9% are naturally occurring.
- Because half the natural chemicals tested are positive, human exposures to rodent carcinogens are likely to be ubiquitous.
- In animal cancer tests, the doses administered are at the maximum tolerated dose (MTD). At the MTD a chemical can cause chronic cell killing and cell replacement in a target tissue which is a cancer risk factor itself.
- In high-dose bioassays, cell division increases mutagenesis and therefore carcinogenesis.
- Extrapolation of cancer potency results from MTD studies to real-life exposures is not scientifically supportable.
- Extrapolation of cancer potency results in rodents to humans cannot be validated.

The key value taken from the CPDB is the $TD_{50}$. This value is the dose rate in mg/kg body wt/day that will induce tumors in half of test animals that otherwise would have remained tumor-free at zero dose (75). A low value of $TD_{50}$ indicates a potent carcinogen and a high value indicates a weak one. In the rodent database that we used for our analysis, data may be present either for mice, rats or both. By convention, the lowest $TD_{50}$ is used for comparison.
To compare various exposures, Ames and co-workers use the term % HERP (Human Exposure to Rodent Potential) that is defined as follows:

$$\%\ \text{HERP} = \frac{\text{Actual Dose Rate, mg/kg/day} \times 100}{TD_{50}\text{, mg/kg/day}}$$

The conventional body weight used in a human comparison is 70 kg. Several important reference points in interpreting % HERP data are the following: 0.00003 (based on rat $TD_{50}$) and 0.00001 (based on mouse) equate to a risk of one in a million, and the background % HERP for the average chloroform level in a liter of US tap water is 0.0003. In Table 4, % HERP ranking of typical US daily human exposures is shown.
As may be seen by the results shown in Table 4, the one-in-a-million risk acceptable to many regulatory agencies is surpassed by every entry in the table except for the rodent carcinogen IQ that originates from eating a fried hamburger. Wine, beer, and coffee drinkers should beware.
Before proceeding with the ranking of MSS toxicants, a word of caution emphasized by GOLD and co-workers (75) needs discussion. Standard practice in regulatory risk assessment for chemicals is to extrapolate high-dose animal data to low-dose human exposure without regards to mechanism. If the mechanism of action were known, it is possible that many of the compounds listed in the CPDB database would not be classified as human carcinogens. For example, *D*-limonene which is listed both in Table 1 as an MSS toxicant and in Table 4 as a rodent carcinogen induces tumors only in male rat kidney tubules with involvement of $alpha_{2u}$-globulin nephrotoxicity. This mechanism does not appear to be possible in humans (76). Therefore, there is no convincing evidence that *D*-limonene is a human carcinogen when its mechanism of action is considered.
For this review we have taken the data from the CPDB and applied it when available to the list of MSS toxicants contained in Table 1. The only assumptions made were that we have a pack-a-day smoker who weighs 70 kg. The results of this analysis are shown in Table 5. As may be seen from the table, only eight of the MSS toxicants for which data are available stand below the one-in-a-million risk category. However, we must keep in mind that just because a compound has a relatively high % HERP score, e.g., *D*-limonene at 0.008403, does not make it a human health hazard.

## 10 SELECTION OF BEST AVAILABLE CARCINOGENIC POTENCY VALUES FOR RANKING MSS TOXICANTS

As mentioned previously, at least two quantitative rankings of MSS toxicants have been made. One ranking by VORHEES *et al.* (31) in 1997 was in support of the Massachusetts Department of Public Health Tobacco Control Program. The other ranking was part of a year 2000 report to the New Zealand Ministry of Health by FOWLES and BATES (33). Both of these reports contain analyses for carcinogenic effects and non-cancer health effects. Analyses for carcinogenic effects rely on Inhalation Unit Risk Factors in units of $(mg/m^3)^{-1}$ as measures of potency. Because some

**Table 4. Possible hazard from daily human exposure of rodent carcinogens**; the subscript to the $TD_{50}$ values refers to either rat (R) or mouse (M) data. [a]

| Human exposure | g/day | Rodent carcinogen | mg/day [b] | $TD_{50}$ in mg/kg | % HERP |
|---|---|---|---|---|---|
| Beer | 257 | ethanol | 11479 | $9110_R$ | 2.1 |
| Wine | 28.0 | ethanol | 3826 | $9110_R$ | 0.5 |
| Home air [c] | | formaldehyde | 0.598 | $2.19_R$ | 0.4 |
| Coffee | 13.3 | caffeic acid | 23.9 | $297_R$ | 0.1 |
| Lettuce | 14.9 | caffeic acid | 7.90 | $297_R$ | 0.04 |
| Black pepper | 0.446 | *D*-limonene | 3.57 | $204_R$ | 0.03 |
| Orange juice | 138 | *D*-limonene | 4.28 | $204_R$ | 0.03 |
| Safrole in spices | | safrole | 1.2 | $51.3_M$ | 0.03 |
| Apple | 32.0 | caffeic acid | 3.40 | $297_R$ | 0.02 |
| Coffee | 13.3 | catechol | 1.33 | $118_R$ | 0.02 |
| Coffee | 13.3 | furfural | 2.09 | $197_M$ | 0.02 |
| Mushroom | 2.55 | hydrazines | | $20300_M$ | 0.02 |
| Cinnamon | 21.9 | coumarin | 0.065 | $13.9_R$ | 0.007 |
| Coffee | 13.3 | hydroquinone | 0.333 | $82.8_R$ | 0.006 |
| Carrot | 12.1 | aniline | 0.624 | $194_R$ | 0.005 |
| Celery | 7.95 | caffeic acid | 0.858 | $297_R$ | 0.004 |
| Potato | 54.9 | caffeic acid | 0.867 | $297_R$ | 0.004 |
| White bread | 67.6 | furfural | 0.500 | $197_M$ | 0.004 |
| Home air [d] | | benzene | 0.155 | $77.5_M$ | 0.003 |
| Nutmeg | 0.0274 | *D*-limonene | 0.466 | $204_R$ | 0.003 |
| Carrot | 12.1 | caffeic acid | 0.374 | $297_R$ | 0.002 |
| Ethylenethiourea [e] | | ethylenethiourea | 0.00951 | $7.9_R$ | 0.002 |
| Pear | 3.29 | caffeic acid | 0.240 | $297_R$ | 0.001 |
| Plum | 2.00 | caffeic acid | 0.276 | $297_R$ | 0.001 |
| Brown mustard | 0.0684 | allyl isothiocyanate | 0.0629 | $96_R$ | 0.0009 |
| Bacon | 11.5 | *N*-nitrosodiethylamine | 0.0000115 | $0.0237_R$ | 0.0007 |
| TCDD [f] | | TCDD | 0.000012 | $0.0000235_R$ | 0.0007 |
| Bacon | 11.5 | *N*-nitrosopyrrolidine | 0.000196 | $0.679_M$ | 0.0004 |
| Bacon | 11.5 | *N*-nitrosodimethylamine | 0.0000345 | $0.124_R$ | 0.0004 |
| Tap water | 1000 | bromodichloromethane | 0.013 | $47.7_M$ | 0.0004 |
| Tap water | 1000 | chloroform | 0.017 | $90.3_M$ | 0.0003 |
| Beer | 257 | furfural | 0.0399 | $197_M$ | 0.0003 |
| PCBs [g] | | PCBs | 0.000098 | $1.74_R$ | 0.00008 |
| Toast | 67.6 | ethyl carbamate {urethane} | 0.000811 | $16.9_M$ | 0.00007 |
| Hamburger | 85 | PhIP | 0.000176 | $4.29_R$ | 0.00006 |
| Hamburger | 85 | MeIQx | 0.0000381 | $1.99_R$ | 0.00003 |
| Beer | 257 | ethyl carbamate {urethane} | 0.000115 | $16.9_M$ | 0.00001 |
| Hamburger | 85 | IQ | 0.00000638 | $1.89_R$ | 0.000005 |

[a] Gold, L.S., T.H. Slone and B.N. Ames: Overview of analyses of the carcinogenic potency database; *in*: Handbook of carcinogenic potency and genotoxicity databases, edited by L.S. Gold and E. Zeiger, CRC Press, Boca Raton, FL, 1997, at http://potency.berkeley.edu/herp.html, downloaded from the Internet on March 24, 2002.
[b] Calculations assume a 70-kg person.
[c] Value assumes a 14-h exposure per day.
[d] Assumes a 14-h daily exposure in a conventional home.
[e] Daily US average for 1990.
[f] 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin daily US average for 1994.
[g] Daily US average over the period 1984–1986.

of these unit risk factors change frequently and different sources have different values for the same chemical compound, we decided to compile a list of these factors from available sources. Once compiled, a selection was made from available values to use in further analysis. Inhalation unit risk values available for MSS toxicants shown in Table 1 are presented in Table 6.

The values are taken from four major resources including the California EPA Office of Environmental Health Hazard Assessment, the US EPA Integrated Risk Information System (IRIS), the US Department of Energy, Office of Environmental Management, Oak Ridge Operations Office: Risk assessment information system (RAIS), and the US EPA: National-scale toxics assessment (NATA) for 1996.

**Table 5. Comparison of pack-a-day smoker intake with HERP Index [a]**. Agents in bold have been included in previous lists of cigarette MSS toxicants, but no longer appear to be relevant. See footnotes of Table 1 for details. A % HERP ranking of 0.00003% (based on a rat $TD_{50}$) or 0.00001% (based on a mouse $TD_{50}$) equates to a risk of 1 in a million.

| Agent | CAS no. | Comp. cig value µg/cig | $TD_{50}$ [b] mg/kg/day | Species rat or mouse | Intake [c] mg/day | Intake/wt [d] mg/kg/day | % HERP |
|---|---|---|---|---|---|---|---|
| Formaldehyde | 50-00-0 | 2.31 E+01 | 2.19 E+00 | R | 4.62 E−01 | 6.60 E−03 | 0.301 |
| Crotonaldehyde | 123-73-9 | 2.18 E+01 | 4.20 E+00 | R | 4.36 E−01 | 6.23 E−03 | 0.148 |
| Acetaldehyde | 75-07-0 | 6.40 E+02 | 1.53 E+02 | R | 1.28 E+01 | 1.83 E−01 | 0.120 |
| 1,3-Butadiene | 106-99-0 | 4.10 E+01 | 1.39 E+01 | M | 8.20 E−01 | 1.17 E−02 | 0.0843 |
| Ethyl carbamate {urethane} | 51-79-6 | 3.80 E+01 | 1.69 E+01 | M | 7.60 E−01 | 1.09 E−02 | 0.0642 |
| Isoprene | 78-79-5 | 4.47 E+02 | 2.74 E+02 | M | 8.94 E+00 | 1.28 E−01 | 0.0466 |
| Nitrobenzene | 98-95-3 | 2.50 E+01 | 2.55 E+01 | R | 5.00 E−01 | 7.14 E−03 | 0.0280 |
| 4-(*N*-Methylnitrosamino)-1-(3-pyridinyl)-1-butanone | 64091-91-4 | 9.70 E−02 | 9.99 E−02 | R | 1.94 E−03 | 2.77 E−05 | 0.0277 |
| Benzene | 71-43-2 | 4.79 E+01 | 7.75 E+01 | M | 9.58 E−01 | 1.37 E−02 | 0.0177 |
| Catechol | 120-80-9 | 4.53 E+01 | 8.47 E+01 | R | 9.06 E−01 | 1.29 E−02 | 0.0153 |
| Hydroquinone | 123-31-9 | 4.29 E+01 | 8.28 E+01 | R | 8.58 E−01 | 1.23 E−02 | 0.0148 |
| Acrylamide | 79-06-1 | 2.20 E+00 | 6.15 E+00 | R | 4.40 E−02 | 6.29 E−04 | 0.0102 |
| Ethylene oxide | 75-21-8 | 7.00 E+00 | 2.13 E+01 | R | 1.40 E−01 | 2.00 E−03 | 0.00939 |
| Styrene {benzene, ethenyl-} | 100-42-4 | 7.60 E+00 | 2.33 E+01 | R | 1.52 E−01 | 2.17 E−03 | 0.00932 |
| *D*-Limonene | 5989-27-5 | 6.00 E+01 | 2.04 E+02 | R | 1.20 E+00 | 1.71 E−02 | 0.00840 |
| *N*-Nitrosoethylmethylamine | 10595-95-6 | 1.30 E−02 | 5.03 E−02 | R | 2.60 E−04 | 3.71 E−06 | 0.00738 |
| *N*-Ntrosodimethylamine | 62-75-9 | 1.91 E−02 | 9.59 E−02 | R | 3.82 E−04 | 5.46 E−06 | 0.00569 |
| *N*-Nitrosopiperidine | 100-75-4 | 2.31 E−01 | 1.30 E+00 | M | 4.62 E−03 | 6.60 E−05 | 0.00508 |
| Hydrazine | 302-01-2 | 4.30 E−02 | 3.09 E−01 | R | 8.60 E−04 | 1.23 E−05 | 0.00398 |
| *N*-Nitrosodiethylamine | 55-18-5 | 2.80 E−03 | 2.65 E−02 | R | 5.60 E−05 | 8.00 E−07 | 0.00302 |
| **DDT** | 50-29-3 | 1.20 E+00 | 1.28 E+01 | M | 2.40 E−02 | 3.43 E−04 | 0.00268 |
| *N*-Nitrosodi-*n*-butylamine | 924-16-3 | 3.00 E−02 | 6.91 E−01 | R | 6.00 E−04 | 8.57 E−06 | 0.00124 |
| **Di(2-ethylhexyl) phthalate** | 117-81-7 | 2.00 E+01 | 6.25 E+02 | R | 4.00 E−01 | 5.71 E−03 | 0.000914 |
| **DDE** | 72-55-9 | 3.70 E−01 | 1.25 E+01 | M | 7.40 E−03 | 1.06 E−04 | 0.000846 |
| Toluene | 108-88-3 | 9.04 E+01 | 3.06 E+03 | R | 1.81 E+00 | 2.58 E−02 | 0.000844 |
| 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TEQ)[e] | 1746-01-6 | 1.26 E−06 | 4.57 E−05 | R | 2.52 E−08 | 3.60 E−10 | 0.000788 |
| Acetamide | 60-35-5 | 3.97 E+00 | 1.80 E+02 | R | 7.94 E−02 | 1.13 E−03 | 0.000630 |
| *N*-Nitrosopyrrolidine | 930-55-2 | 1.40 E−02 | 6.79 E−01 | M | 2.80 E−04 | 4.00 E−06 | 0.000589 |
| PhIP (HCl) [f] | 105650-23-5 | 2.30 E−02 | 1.91 E+00 | R | 4.60 E−04 | 6.57 E−06 | 0.000344 |
| Vinyl acetate | 108-05-4 | 4.00 E+00 | 3.41 E+02 | R | 8.00 E−02 | 1.14 E−03 | 0.000335 |
| Caffeic acid | 331-39-5 | 3.00 E+00 | 2.97 E+02 | R | 6.00 E−02 | 8.57 E−04 | 0.000289 |
| Furfural | 98-01-1 | 1.40 E+00 | 1.97 E+02 | M | 2.80 E−02 | 4.00 E−04 | 0.000203 |
| Carbazole | 86-74-8 | 1.00 E+00 | 1.64 E+02 | M | 2.00 E−02 | 2.86 E−04 | 0.000174 |
| Benzo[*a*]pyrene | 50-32-8 | 5.70 E−03 | 9.56 E−01 | R | 1.14 E−04 | 1.63 E−06 | 0.000170 |
| *N*-Nitrosodi-*n*-propylamine | 621-64-7 | 1.00 E−03 | 1.86 E−01 | R | 2.00 E−05 | 2.86 E−07 | 0.000154 |
| A*α*C | 26148-68-5 | 2.60 E−01 | 4.98 E+01 | R | 5.20 E−03 | 7.43 E−05 | 0.000149 |
| Aniline, 2-methyl- (HCl) [g] | 95-53-4 | 2.00 E−01 | 4.36 E+01 | R | 4.00 E−03 | 5.71 E−05 | 0.000131 |
| Ethylenethiourea | 96-45-7 | 2.70 E−02 | 7.90 E+00 | R | 5.40 E−04 | 7.71 E−06 | 0.000098 |
| Aniline [f] | 62-53-3 | 6.55 E−01 | 2.69 E+02 | R | 1.31 E−02 | 1.87 E−04 | 0.000070 |
| Naphthalene | 91-20-3 | 3.42 E−01 | 1.63 E+02 | M | 6.84 E−03 | 9.77 E−05 | 0.000060 |
| *N'*-Nitrosoanabasine | 37620-20-5 | 2.31 E−02 | 1.19 E+01 | R | 4.62 E−04 | 6.60 E−06 | 0.000055 |
| Biphenyl, 4-amino- | 92-67-1 | 4.00 E−03 | 2.10 E+00 | M | 8.00 E−05 | 1.14 E−06 | 0.000054 |
| MeA*α*C (acetate) [h] | 68006-83-7 | 3.70 E−02 | 2.22 E+01 | R | 7.40 E−04 | 1.06 E−05 | 0.000048 |
| Vinyl chloride | 75-01-4 | 3.00 E−02 | 1.91 E+01 | R | 6.00 E−04 | 8.57 E−06 | 0.000045 |
| ***N*-Nitrosodiethanolamine** | 1116-54-7 | 4.30 E−03 | 3.17 E+00 | R | 8.60 E−05 | 1.23 E−06 | 0.000039 |
| Propylene oxide | 75-56-9 | 1.00 E−01 | 7.44 E+01 | R | 2.00 E−03 | 2.86 E−05 | 0.000038 |
| Trp-P-1 (acetate) [f] | 62450-06-0 | 5.00 E−04 | 5.75 E−01 | R | 1.00 E−05 | 1.43 E−07 | 0.000025 |
| IQ | 76180-96-6 | 3.00 E−04 | 8.12 E−01 | R | 6.00 E−06 | 8.57 E−08 | 0.000011 |
| Naphthalene, 2-amino- | 91-59-8 | 1.11 E−02 | 3.67 E+01 | M | 2.22 E−04 | 3.17 E−06 | 0.000009 |
| Glu-P-1 | 67730-11-4 | 8.90 E−04 | 4.69 E+00 | R | 1.78 E−05 | 2.54 E−07 | 0.000005 |
| Trp-P-2 (acetate) [f] | 62450-07-1 | 1.10 E−03 | 6.66 E+00 | R | 2.20 E−05 | 3.14 E−07 | 0.000005 |

**Table 5 (*cont.*)**

| Agent | CAS no. | Comp. cig value µg/cig | $TD_{50}$ [b] mg/kg/day | Species rat or mouse | Intake [c] mg/day | Intake/wt [d] mg/kg/day | % HERP |
|---|---|---|---|---|---|---|---|
| Dibenz[*a,h*]anthracene | 53-70-3 | 4.00 E−04 | 5.88 E+00 | M | 8.00 E−06 | 1.14 E−07 | 0.000002 |
| MeIQ | 77094-11-2 | 7.50 E−04 | 1.23 E+01 | M | 1.50 E−05 | 2.14 E−07 | 0.000002 |
| Glu-P-2 | 67730-10-3 | 8.80 E−04 | 1.60 E+01 | M | 1.76 E−05 | 2.51 E−07 | 0.000002 |

[a] Gold, L.S., T.H. Slone, and B.N. Ames: Overview of analyses of the carcinogenic potency database; *in*: Handbook of Carcinogenic Potency and Genotoxicity Databases, edited by L.S. Gold and E. Seiger, CRC Press, Boca Raton, FL, 1997, pp. 1–605. Accessed on the Internet, http://potency.berkley.edu/herp.html, March 24, 2002.

[b] Gold, L.S. T.H. Slone, and B.N. Ames: Chapter 3. Summary of carcinogenic potency database by chemical; *in*: Handbook of Carcinogenic Potency and Genotoxicity Databases, edited by L.S. Gold and E. Seiger, CRC Press, Boca Raton, FL, 1997, pp. 621–660. Accessed on the Internet, http://potency.berkeley.edu/txt/crc.chapter3.html, March 24, 2002.

[c] Assumes a pack-a-day smoker, i.e., 20 cig/day.

[d] Assumes that a smoker weighs 70 kg.

[e] In the reference for polychlorodibenzodioxins and polychlorodibenzofurans [Beitr. Tabakforsch. Int. 14 (1990) 393–402] the authors report that the most toxicologically potent isomer of these materials, i.e., 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) is not detected in cigarette mainstream smoke. However, for toxicological comparisons, it is common practice to convert all of the isomers present to their 2,3,7,8-TCDD equivalents. The chlorinated dioxins and benzofurans reported in the article were converted to the toxic equivalents of 2,3,7,8-TCDD with toxic equivalency factors taken from the following source: U.S. EPA: Exposure and human health reassessment of 2,3,7,8-tetracholorodibenzo-*p*-dioxin (TCDD) and related compounds, Part II. Health assessment for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds, Chapt. 9, Toxicity equivalence factors (TEF) for dioxin and related compounds, EPA/600/P-00/001Bb (2000), draft final report, Table 9-1, 9-35.

[f] $TD_{50}$ value is for the HCl salt and has not been adjusted to the free base value.

[g] $TD_{50}$ value is for the HCl salt and has been adjusted to the free base value.

[h] $TD_{50}$ value is for the acetate and has not been adjusted to the free compound.

All of these databases are downloadable from the Internet and the websites are noted in the footnotes of Table 6. When multiple inhalation unit risk values were available, we selected in the following order: the highest value from either the IRIS or RAIS databases, followed by the California EPA value, and followed by the NATA data as our "selected unit risk value" for further cancer risk estimation. Most of the unit risk factors are similar across sources. The cancer risk calculation for $^{210}$Po is different because it is based upon radioactivity emissions and is treated separately.

## 11 CALCULATION OF INCREMENTAL CANCER LIFETIME RISK FOR EXPOSURE TO MSS TOXICANTS

The calculation of incremental cancer lifetime risk for exposure to MSS toxicants follows the procedure and assumptions made by VORHEES *et al.* (31). For each toxicant in Table 1 an average daily concentration (ADC) is calculated according to the following formula:

$$\text{ADC, mg/m}^3 = \frac{\text{Yield per cig, mg} \times \text{Number cig smoked/day}}{\text{Volume of air breathed/day}}$$

where the volume of air breathed per day is assumed to be 20 $m^3$.

The incremental lifetime cancer risk is then calculated as follows:

$$\text{Incremental Lifetime Cancer Risk} = \text{ADC}_{life} \times \text{URF}$$

where:

$ADC_{life}$ = Lifetime Average Daily Concentration ($mg/m^3$)

URF = Unit Risk Factor $(mg/m^3)^{-1}$

The lifetime ADC is estimated by adjusting the daily ADC according to the number of years of smoking (assumed to be 35 years) and the average lifetime (assumed to be 70 years). The equation relating the daily ADC to $ADC_{life}$ is as follows:

$$\text{ADC}_{life} = \frac{\text{ADC} \times \text{Number of years smoking}}{\text{Average lifetime}}$$

We have assumed 35 years of smoking rather than the 30 years used by VORHEES *et al.*

The results of incremental lifetime cancer risk calculations employing yield data from Table 1 and assuming that the person is a pack-a-day smoker are shown in Table 7.

As mentioned earlier, excess incremental lifetime cancer risk (ILCR) for exposure to $^{210}$Po is calculated differently than the other MSS toxicants in Table 7. The calculation is as follows:

$$\begin{aligned}\text{ILCR}_{\text{Po-210}} &= \text{pCi/cig} \times \text{Cig smoked/day} \times \text{Days/year} \\ &\quad \times \text{Number of smoking years} \times \text{risk/pCi} \\ &= 1.60\text{E-02 pCi/cig} \times 20 \text{ cig/day} \times 365 \text{ day/yr} \\ &\quad \times 35 \text{ smoking years} \times 1.08\text{E-08 risk/pCi} \\ &= 4.42\text{E-05}\end{aligned}$$

Thus, the calculated excess lifetime cancer risk for a pack-a-day smoker of 1R4F cigarettes is estimated to be greater than one in a million. However the risk from $^{210}$Po does not appear to be very large. In their comparison of lung cancer incidence in uranium miners exposed to $^{210}$Po vs. cigarette smokers exposed to MSS $^{210}$Po, HARLEY *et al.* (77) questioned the significance of $^{210}$Po in tobacco-induced lung cancer. Their conclusion has often been quoted (7).

**Table 6. Cancer potency values for some toxicants in cigarette mainstream smoke**. Agents in bold have been included in previous lists of cigarette MSS toxicants, but no longer appear to be relevant. See footnotes of Table 1 for details.

| Agent | CAS no. | Cal. EPA inhal. unit risk [a] $(mg/m^3)^{-1}$ | US EPA inhal. unit risk [b] $(mg/m^3)^{-1}$ | NATA 1996 inhal. unit risk [c,d] $(mg/m^3)^{-1}$ | ORNL inhal. unit risk [e] $(mg/m^3)^{-1}$ | Selected inhal. unit risk $(mg/m^3)^{-1}$ |
|---|---|---|---|---|---|---|
| *N*-Nitrosodiethylamine | 55-18-5 | 1.00 E+01 | | | 4.30 E+01 | 4.30 E+01 |
| *N*-Nitrosodimethylamine | 62-75-9 | 4.60 E+00 | | | 1.40 E+01 | 1.40 E+01 |
| Chromium VI | 1333-82-0 | 1.50 E+02 | 1.20 E+01 | | 1.20 E+01 | 1.20 E+01 |
| Dibenzo[*b,def*]chrysene | 189-64-0 | 1.10 E+01 | | | | 1.10 E+01 |
| **Dibenzo[*def,p*]chrysene** | 191-30-0 | 1.10 E+01 | | | | 1.10 E+01 |
| Trp-P-1 | 62450-06-0 | 7.40 E+00 | | | | 7.40 E+00 |
| *N*-Nitrosoethylmethylamine | 10595-95-6 | 6.30 E+00 | | | | 6.30 E+00 |
| Biphenyl, 4-amino- | 92-67-1 | 6.00 E+00 | | | | 6.00 E+00 |
| Hydrazine | 302-01-2 | 4.90 E+00 | 4.90 E+00 | | 4.90 E+00 | 4.90 E+00 |
| Hydrazine, 1,1-dimethyl- | 57-14-7 | | | | 4.90 E+00 | 4.90 E+00 |
| Arsenic | 7440-38-2 | 3.30 E+00 | 4.30 E+00 | | 4.30 E+00 | 4.30 E+00 |
| Quinoline | 91-22-5 | | | 3.40 E+00 | | 3.40 E+00 |
| *N*-Nitrosopiperidine | 100-75-4 | 2.70 E+00 | | | | 2.70 E+00 |
| Propane, 2-nitro- | 79-46-9 | | | | 2.70 E+00 | 2.70 E+00 |
| Beryllium | 7440-41-7 | 2.40 E+00 | 2.40 E+00 | | 2.40 E+00 | 2.40 E+00 |
| *N*-Nitrosodi-*n*-propylamine | 621-64-7 | 2.00 E+00 | | | | 2.00 E+00 |
| Cadmium | 7440-43-9 | 4.20 E+00 | 1.80 E+00 | | 1.80 E+00 | 1.80 E+00 |
| *N*-Nitrosodi-*n*-butylamine | 924-16-3 | 3.10 E+00 | | | 1.60 E+00 | 1.60 E+00 |
| Glu-P-1 | 67730-11-4 | 1.40 E+00 | | | | 1.40 E+00 |
| Acrylamide | 79-06-1 | 1.30 E+00 | | | 1.30 E+00 | 1.30 E+00 |
| **7*H*-Dibenzo[*c,g*]carbazole** | 194-59-2 | 1.10 E+00 | | | | 1.10 E+00 |
| Chrysene, 5-methyl- | 3697-24-3 | 1.10 E+00 | | | | 1.10 E+00 |
| Naphtho[1,2,3,4-*def*]chrysene | 192-65-4 | 1.10 E+00 | | | | 1.10 E+00 |
| Trp-P-2 | 62450-07-1 | 9.10 E−01 | | | | 9.10 E−01 |
| Benzo[*a*]pyrene | 50-32-8 | 1.10 E+00 | | | 8.80 E−01 | 8.80 E−01 |
| Dibenz[*a,h*]anthracene | 53-70-3 | 1.20 E+00 | | | 8.80 E−01 | 8.80 E−01 |
| ***N*-Nitrosodiethanolamine** | 1116-54-7 | 8.00 E−01 | | | | 8.00 E−01 |
| *N*-Nitrosopyrrolidine | 930-55-2 | 6.00 E−01 | | | 6.10 E−01 | 6.10 E−01 |
| Naphthalene, 2-amino- | 91-59-8 | 5.14 E−01 | | | | 5.14 E−01 |
| Glu-P-2 | 67730-10-3 | 4.00 E−01 | | | | 4.00 E−01 |
| IQ | 76180-96-6 | 4.00 E−01 | | | | 4.00 E−01 |
| *N'*-Nitrosonornicotine | 16543-55-8 | 4.00 E−01 | | | | 4.00 E−01 |
| MeA$\alpha$C | 68006-83-7 | 3.40 E−01 | | | | 3.40 E−01 |
| Ethyl carbamate {urethane} | 51-79-6 | 2.90 E−01 | | | | 2.90 E−01 |
| 1,3-Butadiene | 106-99-0 | 1.70 E−01 | 2.80 E−01 | | 2.80 E−01 | 2.80 E−01 |
| Nickel | 7440-02-0 | 2.60 E−01 | 2.40 E−01 | | 2.40 E−01 | 2.40 E−01 |
| A$\alpha$C | 26148-68-5 | 1.14 E−01 | | | | 1.14 E−01 |
| Benzo[*j*]fluoranthene | 205-82-3 | 1.10 E−01 | | | | 1.10 E−01 |
| **Dibenz[*a,h*]acridine** | 226-36-8 | 1.10 E−01 | | | | 1.10 E−01 |
| **Dibenz[*a,j*]acridine** | 224-42-0 | 1.10 E−01 | | | | 1.10 E−01 |
| Ethylene oxide | 75-21-8 | 8.80 E−02 | | | 1.00 E−01 | 1.00 E−01 |
| **DDT** | 50-29-3 | | | | 9.70 E−02 | 9.70 E−02 |
| Benz[*a*]anthracene | 56-55-3 | 1.10 E−01 | | | 8.80 E−02 | 8.80 E−02 |
| Benz[*e*]acephenanthrylene | 205-99-2 | 1.10 E−01 | | | 8.80 E−02 | 8.80 E−02 |
| Indeno[1,2,3-*cd*]pyrene | 193-39-5 | 1.10 E−01 | | | 8.80 E−02 | 8.80 E−02 |
| Acrylonitrile | 107-13-1 | 2.90 E−01 | 6.80 E−02 | | 6.80 E−02 | 6.80 E−02 |
| Aniline, 2-methyl- | 95-53-4 | 5.10 E−02 | | | | 5.10 E−02 |
| 2,3,7,8-TCDD (TEQ) | 1746-01-6 | 3.80 E−02 | | | 3.30 E−02 | 3.30 E−02 |
| Acetamide | 60-35-5 | 2.00 E−02 | | | | 2.00 E−02 |
| Ethylenethiourea | 96-45-7 | 1.30 E−02 | | | | 1.30 E−02 |
| Formaldehyde | 50-00-0 | 6.00 E−03 | 1.30 E−02 | | 1.30 E−02 | 1.30 E−02 |
| Lead | 7439-92-1 | 1.20 E−02 | | | | 1.20 E−02 |
| Benzo[*k*]fluoranthene | 207-08-9 | 1.10 E−01 | | | 8.80 E−03 | 8.80 E−03 |
| Carbazole | 86-74-8 | | | 5.70 E−03 | | 5.70 E−03 |
| Vinyl chloride | 75-01-4 | 7.80 E−02 | 4.40 E−03 | | 8.80 E−03 | 4.40 E−03 |
| Propylene oxide | 75-56-9 | 3.70 E−03 | 3.70 E−03 | | 3.70 E−03 | 3.70 E−03 |

**Table 6 (*cont.*)**

| Agent | CAS no. | Cal. EPA inhal. unit risk [a] $(mg/m^3)^{-1}$ | US EPA inhal. unit risk [b] $(mg/m^3)^{-1}$ | NATA 1996 inhal. unit risk [c,d] $(mg/m^3)^{-1}$ | ORNL inhal. unit risk [e] $(mg/m^3)^{-1}$ | Selected inhal. unit risk $(mg/m^3)^{-1}$ |
|---|---|---|---|---|---|---|
| **Di(2-ethylhexyl) phthalate** | 117-81-7 | 2.40 E−03 | | | | 2.40 E−03 |
| Acetaldehyde | 75-07-0 | 2.70 E−03 | 2.20 E−03 | | 2.20 E−03 | 2.20 E−03 |
| Benzene | 71-43-2 | 2.90 E−02 | 2.20 E−03 | | 7.80 E−03 | 2.20 E−03 |
| Aniline | 62-53-3 | 1.60 E−03 | | | | 1.60 E−03 |
| Chrysene | 218-01-9 | 1.10 E−02 | | | 8.80 E−04 | 8.80 E−04 |
| Polonium-210 (pCi)[f] | 7440-08-6 | | | | 1.08 E−08 [f] | 1.08 E−08 [f] |

[a] California EPA Office of Environmental Health Hazard Assessment/Risk Assessment: California cancer potency values, downloaded as a PDF file on June 6, 2002 from www.oehha.ca.gov/risk/chemicalDB.
[b] US EPA Integrated Risk Information System (IRIS): Inhalation RfCs and air unit risk factors, downloaded on June 6, 2002 from www.epa.gov/iris.
[c] US EPA: National-scale air toxics assessment for 1996, EPA-453/R-01-003 (2001) Appendix H, Table 1, downloaded on June 6, 2002 from www.epa.gov/ttn/atw/nata/natsa4.html.
[d] US EPA: Health effects assessment summary tables, EPA-540-R-97-036 (1997).
[e] US Department of Energy, Office of Environmental Management, Oak Ridge Operations Office: Risk assessment information system, Risk assessment tools, June 2002, nonradionuclides in Excel spreadsheet and radionuclides in Excel spreadsheet, downloaded on June 20, 2002 from http://risk.lsd.ornl.gov/tox/tox_values.shtml.
[f] Inhalation units for $^{210}Po$ are given in risk/pCi.

## 12 QUALITATIVE RANKING OF EXCESS LIFETIME CANCER RISK

To aid the reader in interpretation of the estimated lifetime cancer risk, Table 8 prepared by the NEW YORK STATE DEPARTMENT OF HEALTH (78) is presented.
Additionally, the reference states, "An estimated increased excess lifetime cancer risk is not a specific estimate of expected cancers. Rather, it is a plausible *upper bound estimate* [emphasis added] of the probability that a person may develop cancer sometime in his or her lifetime following exposure to that contaminant."
For the compounds listed in Table 7 that have estimated incremental lifetime cancer risk greater than one in a million, i.e., 1.00E-06, seventeen toxicants use old yield data for nonfiltered cigarettes and two of these seventeen compounds, DDT and *N*-nitrosodiethanolamine, are obsolete MSS toxicants.

## 13 SELECTION OF NON-CANCER HEALTH EFFECTS TOXICITY VALUES FOR RANKING MSS TOXICANTS

Just as there is a variety of sources for cancer potency values, there are multiple sources of data for non-cancer effects. From VORHEES *et al.* (31) we get the following definition:

> The toxicity criteria used to calculate potential non-cancer risk for the inhalation route of exposure are reference concentrations (RfCs). An RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious non-cancer effects during a lifetime (US EPA 1997). The smaller the RfC, the more potent the compound. RfCs are designed to provide conservative estimates of health risk that are protective for the most sensitive members of the population.

The RfC values in $mg/m^3$ were downloaded from two websites, i.e., the US EPA IRIS site and the Oak Ridge National Laboratory (ORNL) RAIS site. Both sources are fully referenced in Table 9. An additional resource was the US EPA 1997 Health Effects Summary Tables (HEAST). For the smoke toxicants listed in Table 1 there are fewer RfCs available than carcinogenic potency values. Most often the same RfC values were contained both in the IRIS and RAIS databases. All the values are shown in the following table as well as our "selected" RfC.

## 14 CALCULATION OF NON-CANCER RISK FROM EXPOSURE TO MSS TOXICANTS

Non-cancer risk potential is calculated by dividing the average daily concentration (ADC) in $mg/m^3$ of a substance by its RfC. The quotient from this division is called the hazard index (HI).

$$\text{Hazard Index} = \frac{\text{ADC, mg/m}^3}{\text{RfC, mg/m}^3}$$

Hazard indices greater than one suggest a potential for adverse health effects while indices less than one indicate that it is unlikely for even a sensitive subpopulation to experience adverse health effects. In our calculations of hazard indices for MSS toxicants, we again assumed a pack-a-day smoker and a total daily breathing volume of 20 $m^3$. Results of our analysis are shown in Table 10.
There were RfC values available for 24 MSS toxicants listed in Table 1. The estimated HI for eight smoke toxicants exceeded the ratio of one. Estimates for two of these compounds, hydrogen sulfide and nitrobenzene, are based upon old MSS yield data. It appears from the data in Table 10 that acrolein has the greatest potential for causing adverse non-cancer health effects.

**Table 7. Estimated chemical specific incremental lifetime cancer risk for a pack-a-day smoker**. Agents in bold have been included in previous lists of cigarette MSS toxicants, but no longer appear to be relevant. See footnotes of Table 1 for details.

| Agent | CAS no. | Comp. cig value µg/cig | ADC [a] 20 cig µg/m$^3$ | $ADC_{life}$ [b] µg/m$^3$ | Inhal. unit risk (mg/m$^3$)$^{-1}$ | Incremental lifetime cancer risk |
|---|---|---|---|---|---|---|
| 1,3-Butadiene | 106-99-0 | 4.10 E+01 | 4.10 E+01 | 2.05 E+01 | 2.80 E−01 | 5.74 E−03 |
| Ethyl carbamate {urethane} | 51-79-6 | 3.80 E+01 | 3.80 E+01 | 1.90 E+01 | 2.90 E−01 | 5.51 E−03 |
| Propane, 2-nitro- | 79-46-9 | 2.20 E+00 | 2.20 E+00 | 1.10 E+00 | 2.70 E+00 | 2.97 E−03 |
| Acrylamide | 79-06-1 | 2.20 E+00 | 2.20 E+00 | 1.10 E+00 | 1.30 E+00 | 1.43 E−03 |
| Acetaldehyde | 75-07-0 | 6.40 E+02 | 6.40 E+02 | 3.20 E+02 | 2.20 E−03 | 7.04 E−04 |
| Quinoline | 91-22-5 | 3.15 E−01 | 3.15 E−01 | 1.58 E−01 | 3.40 E+00 | 5.36 E−04 |
| Acrylonitrile | 107-13-1 | 1.39 E+01 | 1.39 E+01 | 6.95 E+00 | 6.80 E−02 | 4.73 E−04 |
| Ethylene oxide | 75-21-8 | 7.00 E+00 | 7.00 E+00 | 3.50 E+00 | 1.00 E−01 | 3.50 E−04 |
| *N*-Nitrosopiperidine | 100-75-4 | 2.31 E−01 | 2.31 E−01 | 1.16 E−01 | 2.70 E+00 | 3.12 E−04 |
| Formaldehyde | 50-00-0 | 2.31 E+01 | 2.31 E+01 | 1.16 E+01 | 1.30 E−02 | 1.50 E−04 |
| *N*-Nitrosodimethylamine | 62-75-9 | 1.91 E−02 | 1.91 E−02 | 9.55 E−03 | 1.40 E+01 | 1.34 E−04 |
| Hydrazine | 302-01-2 | 4.30 E−02 | 4.30 E−02 | 2.15 E−02 | 4.90 E+00 | 1.05 E−04 |
| Cadmium | 7440-43-9 | 6.73 E−02 | 6.73 E−02 | 3.37 E−02 | 1.80 E+00 | 6.06 E−05 |
| *N*-Nitrosodiethylamine | 55-18-5 | 2.80 E−03 | 2.80 E−03 | 1.40 E−03 | 4.30 E+01 | 6.02 E−05 |
| **DDT** | 50-29-3 | 1.20 E+00 | 1.20 E+00 | 6.00 E−01 | 9.70 E−02 | 5.82 E−05 |
| Benzene | 71-43-2 | 4.79 E+01 | 4.79 E+01 | 2.40 E+01 | 2.20 E−03 | 5.27 E−05 |
| Polonium-210 (pCi) | 7440-08-6 | 1.60 E−02 [c] | 3.20 E−01 [d] | 1.60 E−01 [e] | 1.08 E−08 [f] | 4.42 E−05 [g] |
| *N*-Nitrosoethylmethylamine | 10595-95-6 | 1.30 E−02 | 1.30 E−02 | 6.50 E−03 | 6.30 E+00 | 4.10 E−05 |
| Acetamide | 60-35-5 | 3.97 E+00 | 3.97 E+00 | 1.99 E+00 | 2.00 E−02 | 3.97 E−05 |
| *N*-Nitrosodi-*n*-butylamine | 924-16-3 | 3.00 E−02 | 3.00 E−02 | 1.50 E−02 | 1.60 E+00 | 2.40 E−05 |
| **Di(2-ethylhexyl) phthalate** | 117-81-7 | 2.00 E+01 | 2.00 E+01 | 1.00 E+01 | 2.40 E−03 | 2.40 E−05 |
| *N'*-Nitrosonornicotine | 16543-55-8 | 1.15 E−01 | 1.15 E−01 | 5.75 E−02 | 4.00 E−01 | 2.30 E−05 |
| A*α*C | 26148-68-5 | 2.60 E−01 | 2.60 E−01 | 1.30 E−01 | 1.14 E−01 | 1.48 E−05 |
| Arsenic | 7440-38-2 | 5.80 E−03 | 5.80 E−03 | 2.90 E−03 | 4.30 E+00 | 1.25 E−05 |
| Biphenyl, 4-amino- | 92-67-1 | 4.00 E−03 | 4.00 E−03 | 2.00 E−03 | 6.00 E+00 | 1.20 E−05 |
| Chromium VI | 1333-82-0 | 1.32 E−03 | 1.32 E−03 | 6.60 E−04 | 1.20 E+01 | 7.92 E−06 |
| MeA*α*C | 68006-83-7 | 3.70 E−02 | 3.70 E−02 | 1.85 E−02 | 3.40 E−01 | 6.29 E−06 |
| Aniline, 2-methyl- | 95-53-4 | 2.00 E−01 | 2.00 E−01 | 1.00 E−01 | 5.10 E−02 | 5.10 E−06 |
| *N*-Nitrosopyrrolidine | 930-55-2 | 1.40 E−02 | 1.40 E−02 | 7.00 E−03 | 6.10 E−01 | 4.27 E−06 |
| Chrysene, 5-methyl- | 3697-24-3 | 7.60 E−03 | 7.60 E−03 | 3.80 E−03 | 1.10 E+00 | 4.18 E−06 |
| Naphthalene, 2-amino- | 91-59-8 | 1.11 E−02 | 1.11 E−02 | 5.55 E−03 | 5.14 E−01 | 2.85 E−06 |
| Carbazole | 86-74-8 | 1.00 E+00 | 1.00 E+00 | 5.00 E−01 | 5.70 E−03 | 2.85 E−06 |
| Benzo[*a*]pyrene | 50-32-8 | 5.70 E−03 | 5.70 E−03 | 2.85 E−03 | 8.80 E−01 | 2.51 E−06 |
| Trp-P-1 | 62450-06-0 | 5.00 E−04 | 5.00 E−04 | 2.50 E−04 | 7.40 E+00 | 1.85 E−06 |
| ***N*-Nitrosodiethanolamine** | 1116-54-7 | 4.30 E−03 | 4.30 E−03 | 2.15 E−03 | 8.00 E−01 | 1.72 E−06 |
| Benzo[*j*]fluoranthene | 205-82-3 | 2.10 E−02 | 2.10 E−02 | 1.05 E−02 | 1.10 E−01 | 1.16 E−06 |
| *N*-Nitrosodi-*n*-propylamine | 621-64-7 | 1.00 E−03 | 1.00 E−03 | 5.00 E−04 | 2.00 E+00 | 1.00 E−06 |
| Nickel | 7440-02-0 | 5.58 E−03 | 5.58 E−03 | 2.79 E−03 | 2.40 E−01 | 6.70 E−07 |
| Glu-P-1 | 67730-11-4 | 8.90 E−04 | 8.90 E−04 | 4.45 E−04 | 1.40 E+00 | 6.23 E−07 |
| Beryllium | 7440-41-7 | 5.00 E−04 | 5.00 E−04 | 2.50 E−04 | 2.40 E+00 | 6.00 E−07 |
| Benz[*a*]anthracene | 56-55-3 | 1.24 E−02 | 1.24 E−02 | 6.20 E−03 | 8.80 E−02 | 5.46 E−07 |
| Aniline | 62-53-3 | 6.55 E−01 | 6.55 E−01 | 3.28 E−01 | 1.60 E−03 | 5.24 E−07 |
| Trp-P-2 | 62450-07-1 | 1.10 E−03 | 1.10 E−03 | 5.50 E−04 | 9.10 E−01 | 5.01 E−07 |
| **7*H*-Dibenzo[*c,g*]carbazole** | 194-59-2 | 7.00 E−04 | 7.00 E−04 | 3.50 E−04 | 1.10 E+00 | 3.85 E−07 |
| Benz[*e*]acephenanthrylene | 205-99-2 | 5.50 E−03 | 5.50 E−03 | 2.75 E−03 | 8.80 E−02 | 2.42 E−07 |
| Lead | 7439-92-1 | 3.91 E−02 | 3.91 E−02 | 1.96 E−02 | 1.20 E−02 | 2.35 E−07 |
| Propylene oxide | 75-56-9 | 1.00 E−01 | 1.00 E−01 | 5.00 E−02 | 3.70 E−03 | 1.85 E−07 |
| Dibenz[*a,h*]anthracene | 53-70-3 | 4.00 E−04 | 4.00 E−04 | 2.00 E−04 | 8.80 E−01 | 1.76 E−07 |
| Glu-P-2 | 67730-10-3 | 8.80 E−04 | 8.80 E−04 | 4.40 E−04 | 4.00 E−01 | 1.76 E−07 |
| Ethylenethiourea | 96-45-7 | 2.70 E−02 | 2.70 E−02 | 1.35 E−02 | 1.30 E−02 | 1.76 E−07 |
| Indeno[1,2,3-*cd*]pyrene | 193-39-5 | 3.50 E−03 | 3.50 E−03 | 1.75 E−03 | 8.80 E−02 | 1.54 E−07 |
| **Dibenz[*a,j*]acridine** | 224-42-0 | 2.72 E−03 | 2.72 E−03 | 1.36 E−03 | 1.10 E−01 | 1.50 E−07 |
| Vinyl chloride | 75-01-4 | 3.00 E−02 | 3.00 E−02 | 1.50 E−02 | 4.40 E−03 | 6.60 E−08 |
| IQ | 76180-96-6 | 3.00 E−04 | 3.00 E−04 | 1.50 E−04 | 4.00 E−01 | 6.00 E−08 |
| Chrysene | 218-01-9 | 1.36 E−02 | 1.36 E−02 | 6.80 E−03 | 8.80 E−04 | 5.98 E−09 |

**Table 7 (*cont*.)**

| Agent | CAS no. | Comp. cig value µg/cig | ADC [a] 20 cig µg/m$^3$ | $ADC_{life}$ [b] µg/m$^3$ | Inhal. unit risk (mg/m$^3$)$^{-1}$ | Incremental lifetime cancer risk |
|---|---|---|---|---|---|---|
| Benzo[*k*]fluoranthene | 207-08-9 | 1.30 E−03 | 1.30 E−03 | 6.50 E−04 | 8.80 E−03 | 5.72 E−09 |
| **Dibenz[*a,h*]acridine** | 226-36-8 | 1.00 E−04 | 1.00 E−04 | 5.00 E−05 | 1.10 E−01 | 5.50 E−09 |
| 2,3,7,8-TCDD (TEQ) | 1746-01-6 | 1.26 E−06 | 1.26 E−06 | 6.30 E−07 | 3.30 E−02 | 2.08 E−11 |

[a] Calculation of average daily concentration (ADC) assumes smoking 20 cig/day and a breathing volume of 20 m$^3$. ADC = comparison cig value, µg/cig × 20 cig/day ÷ breathing volume, 20 m$^3$.
[b] The lifetime average daily exposure ($ADC_{life}$) is calculated as follows: $ADC_{life}$, µg/m$^3$ = ADC × 35 years smoking ÷70 year average lifetime.
[c] The comparison cigarette value for $^{210}$Po has units of picocuries per cigarette.
[d] The ADC for $^{210}$Po has units of picocuries per day.
[e] The $ADC_{life}$ for $^{210}$Po is calculated as follows: $ADC_{life}$ = 3.20E−01 pCi/day × 365 day/yr × 35 smoking years and has units of pCi.
[f] The Unit Risk (morbidity) for $^{210}$Po has units of risk/pCi.
[g] The estimated incremental lifetime cancer risk $ILCR_{Po-210}$ from $^{210}$Po is calculated as follows: $ILCR_{Po-210}$ = 1.60E−02 pCi/cig × 20 cig/day × 365 day/yr × 35 smoking years × 1.08E−08 risk/pCi.

**Table 8. Qualitative ranking of excess lifetime cancer risk**

| Risk ratio | Qualitative descriptor |
|---|---|
| Equal to or less than one in a million | very low |
| Greater than one in a million to less than one in ten thousand | low |
| One in ten thousand to less than one in a thousand | moderate |
| One in a thousand to less than one in ten | high |
| Equal to or greater than one in ten | very high |

## 15 THE ASSERTION OF THE GENERATION OF TOXICANTS FROM ADDITIVES

In a previous section of our paper, we touched briefly on the assertions that tobacco additives are a source of toxicants and should be investigated accordingly. Having achieved greater "tar" reduction than the cigarette-smoking critics had originally proposed, e.g., see WYNDER (36), the Tobacco Industry unwittingly provided an alternate subject for criticism. The late 1970s, early 1980s heralded the advent of low-"tar" and ultralow-"tar" cigarettes and their acquisition of a significant share of the US cigarette market. Bases of the criticism were a) some commercial low-"tar" brands might have levels of additives much higher than the levels in previous high- and medium-"tar" cigarettes and b) the fates of many of the individual added components during the cigarette smoking process were unknown.

In the 1950s, concern was expressed about the pyrogenesis of PAHs from tobacco components (36) and their possible pyrogenesis from additives. However, the proponents of possible problems with tobacco additives became much more vocal about them when the nearly 70% reduction in sales-weighted MSS "tar" delivery between 1955 and 1985 not only answered the criticisms voiced in the late 1950s, early 1960s but met the goal set by others, i.e., the halving of "tar" delivery as a means to lower lung cancer incidence in cigarette smokers (36).

In 1980, LAVOIE *et al*. (79) wrote:

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

Despite their criticism of the possible increased use of flavorants in the filler of low-"tar", low-nicotine cigarettes, a key part of this discussion is the admission by LAVOIE *et al*. that prior to 1980, the US cigarette manufacturers had apparently achieved a "reduction of toxic and tumorigenic effects in the smoke of low-"tar," low nicotine cigarettes". In the 1979 report of the SURGEON GENERAL [see pp. 63–64 in (43)] the following was written:

> [T]he trend toward low-tar, low-nicotine cigarettes and toward a reduction of undesirable volatile smoke compounds has brought about major changes in the smoke flavor of cigarettes. The use of rolled stems and reconstituted tobacco sheet admixed with leaf lamina and the use of effective filter tips are major factors inducing changes in smoke flavor. All of these developments have led to increased use of flavor additives, especially for low-tar, low-nicotine cigarettes. In fact, these new cigarettes require flavor corrections by additives in order to be acceptable to the consumer. Tobacco extracts as well as nontobacco flavors, such as licorice, cocoa, fruit, spices, and floral compositions, are used . . . At present, the selection of tobacco flavor additives from the GRAS (Generally Regarded As Safe) List or from natural extracts and the screening of their smoke decomposition products for toxicity or other biological activity are not required by law and are done voluntarily by manufacturers.

Critics then asserted that the Industry's use of higher levels of flavorants in low-"tar" cigarettes might increase the hazard to the smoker because the fate of the added ingredients was not known [see RODGMAN (16)]. No evidence was ever presented that the added flavorants actually increased the risk.

**Table 9. Non-cancer reference concentrations for some toxicants in cigarette mainstream smoke**

| Agent | CAS no. | EPA RfC [a] mg/m$^3$ | HEAST RfC [b] mg/m$^3$ | ORNL RfC [c] mg/m$^3$ | Selected RfC mg/m$^3$ |
|---|---|---|---|---|---|
| 2-Butanone | 78-93-3 | | 1.00 E+00 | 1.00 E+00 | 1.00 E+00 |
| Methanol | 67-56-1 | 1.00 E+00 | | | 1.00 E+00 |
| Styrene {benzene, ethenyl-} | 100-42-4 | 1.00 E+00 | | | 1.00 E+00 |
| Carbon disulfide | 75-15-0 | 7.00 E−01 | | 7.00 E−01 | 7.00 E−01 |
| Toluene | 108-88-3 | 4.00 E−01 | | 4.00 E−01 | 4.00 E−01 |
| Vinyl acetate | 108-05-4 | 2.00 E−01 | | 2.00 E−01 | 2.00 E−01 |
| Ammonia | 7664-41-7 | 1.00 E−01 | | 1.00 E−01 | 1.00 E−01 |
| Vinyl chloride | 75-01-4 | 1.00 E−01 | | 1.00 E−01 | 1.00 E−01 |
| Acetonitrile | 75-05-8 | 6.00 E−02 | | 6.00 E−02 | 6.00 E−02 |
| Furfural | 98-01-1 | | | 5.00 E−02 | 5.00 E−02 |
| Propylene oxide | 75-56-9 | 3.00 E−02 | | 3.00 E−02 | 3.00 E−02 |
| Propane, 2-nitro- | 79-46-9 | 2.00 E−02 | | 2.00 E−02 | 2.00 E−02 |
| Acetaldehyde | 75-07-0 | 9.00 E−03 | | 9.00 E−03 | 9.00 E−03 |
| Hydrogen cyanide | 74-90-8 | 3.00 E−03 | | 3.00 E−03 | 3.00 E−03 |
| Naphthalene | 91-20-3 | 3.00 E−03 | | 3.00 E−03 | 3.00 E−03 |
| Acrylonitrile | 107-13-1 | 2.00 E−03 | | 2.00 E−03 | 2.00 E−03 |
| Nitrobenzene | 98-95-3 | | | 2.00 E−03 | 2.00 E−03 |
| Aniline | 62-53-3 | 1.00 E−03 | | 1.00 E−03 | 1.00 E−03 |
| Hydrogen sulfide | 7783-06-4 | 1.00 E−03 | | 1.00 E−03 | 1.00 E−03 |
| Hydroquinone | 123-31-9 | | 1.00 E−03 | | 1.00 E−03 |
| Mercury | 7439-97-6 | 3.00 E−04 | | 3.00 E−04 | 3.00 E−04 |
| Acrolein | 107-02-8 | 2.00 E−05 | | 2.00 E−05 | 2.00 E−05 |
| Beryllium | 7440-41-7 | 2.00 E−05 | | | 2.00 E−05 |
| Chromium VI | 1333-82-0 | 8.00 E−06 | | 1.00 E−04 | 8.00 E−06 |

[a] US EPA Integrated Risk Information System (IRIS): Inhalation RfCs and air unit risk factors, downloaded on June 6, 2002 from www.epa.gov/iris.
[b] US EPA: Health effects assessment summary tables, EPA-540-R-97-036.
[c] US Department of Energy, Office of Environmental Management, Oak Ridge Operations Office: Risk assessment information system, Risk assessment tools, June 2002 nonradionuclides in Excel spreadsheet, downloaded on June 20, 2002 from http://risk.lsd.ornl.gov/tox/tox_values.shtml.

**Table 10. Estimated chemical specific non-cancer risks for a pack-a-day smoker**

| Agent | CAS no. | Comp. cig value µg/cig | ADC [a] 20 cig µg/m$^3$ | Inhal. RfC mg/m$^3$ | Hazard index [b] |
|---|---|---|---|---|---|
| Acrolein | 107-02-8 | 6.50 E+01 | 6.50 E+01 | 2.00 E−05 | 3250.0000 |
| Hydrogen sulfide | 7783-06-4 | 9.00 E+01 | 9.00 E+01 | 1.00 E−03 | 90.0000 |
| Acetaldehyde | 75-07-0 | 6.40 E+02 | 6.40 E+02 | 9.00 E−03 | 71.1111 |
| Hydrogen cyanide | 74-90-8 | 1.65 E+02 | 1.65 E+02 | 3.00 E−03 | 55.0000 |
| Hydroquinone | 123-31-9 | 4.29 E+01 | 4.29 E+01 | 1.00 E−03 | 42.9000 |
| Nitrobenzene | 98-95-3 | 2.50 E+01 | 2.50 E+01 | 2.00 E−03 | 12.5000 |
| Acrylonitrile | 107-13-1 | 1.39 E+01 | 1.39 E+01 | 2.00 E−03 | 6.9500 |
| Acetonitrile | 75-05-8 | 1.00 E+02 | 1.00 E+02 | 6.00 E−02 | 1.6667 |
| Aniline | 62-53-3 | 6.55 E−01 | 6.55 E−01 | 1.00 E−03 | 0.6550 |
| Toluene | 108-88-3 | 9.04 E+01 | 9.04 E+01 | 4.00 E−01 | 0.2260 |
| Methanol | 67-56-1 | 1.80 E+02 | 1.80 E+02 | 1.00 E+00 | 0.1800 |
| Chromium VI | 1333-82-0 | 1.32 E−03 | 1.32 E−03 | 8.00 E−06 | 0.1650 |
| Ammonia | 7664-41-7 | 1.60 E+01 | 1.60 E+01 | 1.00 E−01 | 0.1600 |
| Naphthalene | 91-20-3 | 3.42 E−01 | 3.42 E−01 | 3.00 E−03 | 0.1140 |
| Propane, 2-nitro- | 79-46-9 | 2.20 E+00 | 2.20 E+00 | 2.00 E−02 | 0.1100 |
| 2-Butanone | 78-93-3 | 9.00 E+01 | 9.00 E+01 | 1.00 E+00 | 0.0900 |
| Furfural | 98-01-1 | 1.40 E+00 | 1.40 E+00 | 5.00 E−02 | 0.0280 |
| Beryllium | 7440-41-7 | 5.00 E−04 | 5.00 E−04 | 2.00 E−05 | 0.0250 |
| Vinyl acetate | 108-05-4 | 4.00 E+00 | 4.00 E+00 | 2.00 E−01 | 0.0200 |
| Mercury | 7439-97-6 | 5.96 E−03 | 5.96 E−03 | 3.00 E−04 | 0.0199 |
| Styrene {benzene, ethenyl-} | 100-42-4 | 7.60 E+00 | 7.60 E+00 | 1.00 E+00 | 0.0076 |
| Propylene oxide | 75-56-9 | 1.00 E−01 | 1.00 E−01 | 3.00 E−02 | 0.0033 |
| Carbon disulfide | 75-15-0 | 2.00 E+00 | 2.00 E+00 | 7.00 E−01 | 0.0029 |
| Vinyl chloride | 75-01-4 | 3.00 E−02 | 3.00 E−02 | 1.00 E−01 | 0.0003 |

[a] Calculation of average daily concentration (ADC) assumes smoking 20 cig/day and a breathing volume of 20 m$^3$. ADC = comparison cig value, µg/cig x 20 cig/day ÷ breathing volume, 20 m$^3$.
[b] The potential for non-cancer health effects is evaluated by comparing the ADC to the RfC. This ratio of exposure concentration to toxicity reference concentration is termed a hazard index (HI). HI = ADC, µg/m$^3$ x mg/1,000 µg ÷ RfC, mg/m$^3$.

Examination of extensive laboratory data collected during the past four decades, particularly considerable unpublished data generated between the mid-1950s and the late 1970s, indicates that none of the materials used as flavorants on smoking tobacco products, particularly cigarettes marketed by a US manufacturer, imparts any significant adverse chemical or biological properties to the MSS from flavorant-treated tobacco, a conclusion reached by DOULL *et al*. (80) in their recent assessment of available information on nearly 600 ingredients variously used as cigarette tobacco additives in the US Tobacco Industry. Of these ingredients 460 are individual compounds, many of which have been identified in tobacco and/or smoke. Much evidence has been collected to show that the added ingredients do not adversely affect the MSS properties. The evidence includes chemical data, e.g., smoke composition and pyrolysates, biological data on inhalation, skin painting, and genotoxicity.

In more recent detailed assessments of reported chemical and biological properties for the MSSs from cigarettes fabricated with tobacco with or without one or more additives, PASCHKE *et al*. (81) and RODGMAN (16) reached a similar conclusion: No significant increase in the biological activity of tobacco was reported from cigarettes containing added ingredients.

Information that flavorful components in tobacco did not enhance the PAH level in MSS was provided by the study of the organic solvent extraction of tobacco. Ultimately incorporated into the process was an aqueous alcohol-hexane partition step to separate polar, more flavorful tobacco components from the lipophilic components eventually shown to be PAH precursors. When an appropriate portion of the aqueous ethanol-soluble fraction (AEF) was returned to the extracted tobacco, no difference was found in the PAH levels in the MSSs from the extracted tobacco and the AEF-treated tobacco (RODGMAN, 16,34,82). In the mid-1950s, the identities of most polar components were unknown though it was suspected they contributed significantly to MSS flavor and aroma. No adequate fractionation system to separate highly polar compounds in a complex mixture was available, but that situation was resolved in the 1970s. With the capability to isolate and identify highly polar and volatile components of tobacco and its MSS, it was obvious that many were identical with or similar to ingredients of flavor formulations added to specific tobacco blends to impart unique smoking characteristics (DOULL *et al*., 80).

Although chemical data for the pyrogenesis of allegedly harmful smoke components from flavorants added to the blend at microgram levels are generally not available because of the limitations of analytical methodology, indirect confirmation of the effect of such additives on at least one MSS property is available; namely, the effect of addition of a total flavor formulation to the tobacco blend on the mutagenicity, as measured in the Ames *Salmonella typhimurium* test system, of the MSS particulate matter collected on a Cambridge filter pad. It has also been shown that added flavorful ingredients do not have any significant adverse effect on the composition of MSS (21).

For many years, considerable thought was given to development of an accurate analytical method to determine the contribution of trace levels (a few μg/g of tobacco blend) of a flavorant added to cigarette tobacco to the levels of toxicants in MSS. Limitations in analytical methodology precluded the design of an experiment whose results would be meaningful. Even studies with radiolabeled compounds had their limitations in the study of the pyrogenesis of MSS components (cf. SCHMELTZ *et al*., 83).

With the advent of the Ames test in the early 1970s, an alternate to the almost insurmountable task of studying individually the effect of hundreds of flavorants added to cigarette products was devised in an attempt to show the effect on smoke condensate specific mutagenicity of additives used in commercial brands. Such flavor formulations are qualitatively and quantitatively unique for each RJRT commercial brand and comprise many different individual ingredients. This is probably true for commercial brands from other manufacturers. The weight of flavorants added to the RJRT brands ranged from 1.0 to 1.5 mg/g of tobacco blend. Four sets of cigarettes for each of five commercial brands were fabricated. The levels of flavorants, casing materials, and humectants were varied as shown later in this section.

The MSS TPM from each of these 20 cigarette variations was examined for mutagenicity in the Ames test (TA1538 and TA98 strains of *Salmonella typhimurium*) by a contract laboratory.

| Cigarette variation [a] | Flavorant formulation level | Casing materials [b] and humectants [c] level |
|---|---|---|
| A | usual level used on brand | usual level used on brand |
| B | ten times the usual level used on brand | 0 |
| C | 0 | usual level used on brand |
| D | 0 | 0 |

[a] Five cigarette brands included four commercial filter-tipped brands ranging from high- to ultralow-FTC "tar" deliveries plus a commercial mentholated filter-tipped cigarette. All cigarettes were manufactured in 1977.

[b] Licorice, cocoa, and sugars.

[c] Glycerol and propylene glycol.

Because the response of the *Salmonella typhimurium* was linear from 0 to 500 μg/plate of added wet total particulate matter (WTPM), mutagenicity in revertant/plate was tabulated for the WTPM dose level of μg/plate. This permitted comparison of the four cigarette variations for each *Salmonella typhimurium* strain and for each of five commercial brands. It was concluded (84):

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

The results of this study are presented in detail in RODGMAN (16,82).

Substantial amounts of humectants (glycerol, propylene glycol, and/or triethylene glycol) added to tobacco blends are transferred to MSS TPM and sidestream smoke (SSS) TPM (85). Analysis of humectants in MSS indicated that the FTC "tar" from commercial cigarettes contains signifi-

cant amounts of humectants (86). Thus, it is not surprising that their removal from the additive system produces TPM with increased mutagenicity (84). The nonmutagenic humectants act as diluents for the MSS TPM toxicants produced pyrogenetically or transferred directly from tobacco to smoke during smoking. Recently, GAWORSKI *et al*. reported that inhalation of MSS from cigarettes with glycerol and propylene glycol, added either individually or in combination, had no significant adverse biological effects on rats (87).

It is apparent that the flavorants used in the commercial brands studied do not increase its MSS specific mutagenicity. In fact, flavorant removal increases slightly the observed mutagenicity of the WTPM. The findings from this study indicate that the additives in the flavorant formulations for five commercial products do not contribute toxicants to the smoke whose levels and potency are such that they produce abnormal increases in the specific mutagenicity as measured in the Ames test system.

To the knowledge gained in the 1950s on the effect of added compounds on the chemical composition of MSS, particularly its PAH content (16,82) and in the 1970s on the effect of product flavor formulations on MSS specific mutagenicity (84) was recently added even more definitive knowledge on the effect of addition of a mixture of selected ingredients to cigarette tobacco on laboratory animals a) exposed to the resulting MSS by inhalation and b) treated via skin painting with the resulting CSC.

Among flavorants, menthol is special because its usage level is several magnitudes greater than that of any other component in the flavor formulation. Chemically, its fate during smoking was defined by NEWELL *et al*. (88) and JENKINS *et al.* (89) from studies with $^{14}$C-menthol. Less than 2% of the added menthol undergoes pyrolysis during smoking. Biologically, added menthol produces little change in the effects: a) In 1965, BOCK *et al*. (90) reported no difference between the specific tumorigenicities of CSCs from non-mentholated vs. mentholated cigarettes. b) A 10-fold increase in the levels of the flavorant formulation and menthol on a commercial cigarette blend produced no significant change in specific mutagenicity (84). c) In a 13-week inhalation study with rats, GAWORSKI *et al.* (17) reported that addition of 5000 ppm of menthol to the blend had no substantial effect on the character or extent of the biological responses normally associated with inhalation of cigarette MSS.

Almost 77% of the items listed by DOULL *et al*. (80) as ingredients added by the six major US cigarette manufacturers during cigarette production are individual compounds, the remaining items are mixtures, e.g., natural oils, plant extracts, oleoresins. As noted previously by DOULL *et al*. (80); GAWORSKI *et al*. (18); PASCHKE *et al*. (81); RUSTEMEIER *et al*. (21); and RODGMAN (16), the compounds may fall into one of the following categories: a) It is a component of one or more of the tobacco types [flue-cured (LLOYD *et al*., 91); burley (ROBERTS and ROHDE, 92); Oriental (SCHUMACHER and VESTAL, 93); Maryland (SCHUMACHER, 94)] commonly used in cigarette blends. b) It is a component of cigarette MSS (80). c) It is a component of both tobacco and tobacco smoke. d) It is a homolog or isomer of an identified tobacco and/or tobacco smoke component.

In their study of added ingredients on the biological effect of inhaled cigarette MSS, GAWORSKI *et al*. (18) administered to rats via inhalation the MSS from cigarettes to which 172 ingredients (129 individual compounds, 43 mixtures) had been added. Most of the ingredients are included in the DOULL *et al.* list. From the results of their inhalation experiment, GAWORSKI *et al*. concluded:

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

In a similar biological study, GAWORSKI *et al*. (19) investigated the effect on the specific tumorigenicity of the CSC from cigarettes to which 150 ingredients (109 individual compounds, 41 mixtures) had been added. Here again, most ingredients are included in the DOULL *et al.* list. From the results of their skin-painting study, GAWORSKI *et al*. concluded:

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

In early 2002, four reports by CARMINES and colleagues (20–23) were published on their excellent study of the effects of ingredients added to a cigarette on the chemical and biological properties of its MSS. A total of 333 ingredients commonly used in cigarette manufacture was added to a test cigarette, representative of a commercial blended cigarette. Ingredients were added at approximately the levels normally used in commercial cigarettes and at levels several times those normally used. The MSS data vs. those from a control cigarette with no added ingredients indicated an increase in the TPM. Normalizing the yields of individual MSS ingredients to the TPM yields indicated a reduction in the majority of them. An increase in the amount relative to TPM was observed for only a few MSS components (RUSTEMEIER *et al*., 21). These chemical results on the MSSs are consistent with the results obtained not only in *in vitro* mutagenicity and cytotoxicity studies with the TPMs from the ingredient-treated and control cigarettes (ROEMER *et al*., 22) but also in *in vivo* studies with rats exposed via inhalation to the MSSs from the treated and control cigarettes (VANSCHEEUWIJCK *et al*., 23): The addition of the ingredients did not increase the *in vitro* mutagenicity or cytotoxicity of the TPMs from the ingredient-treated cigarettes or the inhalation toxicity to rats of their MSSs even at the exaggerated exposure level used.

These findings not only bolster the observations reported by RODGMAN (16) but also the conclusions reached by DOULL *et al*. (80), PASCHKE *et al*. (81), and GAWORSKI *et al*., 17–19,87) on the effect of added ingredients listed by DOULL *et al*. on the chemical and biological properties of cigarette MSS.

Over the years it has been repeatedly asserted [see (16)] that cigarette ingredients added at normal levels to pre-1980 cigarettes or at slightly increased levels to more recent lower "tar" cigarettes might adversely modify the chemistry and biology of the MSSs from such cigarettes. However, no chemical or biological evidence has been presented in support of such assertions.

## 16 INHIBITORS AND ANTICARCINOGENS IN CIGARETTE MSS

In the preceding sections, a) the listing of numerous MSS components as significant toxicants is questioned and b) the assertions that ingredients added to cigarette tobacco adversely affect the chemical and biological properties of MSS are shown to be in error. In this section we will discuss the identified MSS components that have been shown in bioassays to significantly diminish the adverse biological effects of a number of the listed MSS toxicants.

The toxicological properties of a MSS component asserted to adversely affect the smoker have generally been defined in one or more bioassays devoted to the study of the effect of the component administered individually to a host. In most cases other than numerous studies of tumorigenesis, the effect on the toxicological property of a specific compound by other compounds such as those in the complex MSS aerosol has not been studied. The toxicological effect of a specific component in MSS is usually derived by extrapolation from the effect observed in one or more bioassays with the individual component.

It is known that the complex MSS aerosol has a significant effect on the chemistry of components in it. For example, a) the rate of conversion of NO to $NO_2$ is significantly different in the MSS aerosol than in a system comprising only NO and $O_2$ (95–97) and b) methyl nitrite reported as an MSS component is not formed during the smoking process but is formed during ageing of the MSS during the analytical procedure (98). If the chemistry of an MSS aerosol component be altered by the presence of thousands of other aerosol components, then logic dictates that its toxicology will also be altered.

Except for tumorigenic effects, little has been reported on the effect of other components in the complex MSS aerosol on the toxicological properties of an individual component. The tumorigenicity of many MSS components has been discussed frequently and in great detail but little has been written about the biological activity of nontumorigenic MSS components reported to counteract the tumorigenicity in laboratory animals of the various tumorigens.

In 1941, SHEAR and LEITER (99) described in detail the many factors affecting tumorigenicity of a chemical. In the mid-1940s, several nontumorigenic aromatic hydrocarbons (benzene, naphthalene, anthracene) administered with B*a*P or dibenz[*a,h*]anthracene (DBA) significantly diminished the B*a*P and DBA tumorigenicity (100). In recent lists of MSS toxicants, benzene, B*a*P, and DBA are listed as significant tumorigens. Reported many times, however, is the noncarcinogenicity of benzene in the solvent-control group when it was used as the solvent for known or suspect tumorigens in skin-painting bioassays (101,102).

STEINER and FALK (103) reported that benz[*a*]anthracene (B*a*A), categorized as either an extremely weak or an inactive mouse-skin tumorigen (104), significantly diminishes DBA tumorigenicity when both DBA and B*a*A are administered simultaneously by subcutaneous injection. Despite this and similar bioassay results plus the presence of B*a*A and DBA in MSS, both are repeatedly categorized as significant tumorigens in cigarette MSS! Similar inhibition was reported with mixtures of 7,12-dimethylbenz[*a*]anthracene (DMBA) and several inactive PAHs (105).

In subsequent studies, other nontumorigenic PAHs (phenanthrene, fluoranthene, pyrene) were reported to be effective antitumorigens against B*a*P and DMBA (106,107). The nontumorigenic hydrocarbons – benzene, naphthalene, anthracene, phenanthrene, fluoranthene, pyrene – are MSS components, present at per cigarette delivery levels far in excess of those of B*a*P, DBA, or any of the other PAHs classified as tobacco smoke toxicants.

Much evidence collected since 1932 on the tumorigenicity of PAHs indicates their tumorigenicity is not inherent but depends on specific metabolites that comprise one or more epoxides, dihydroxy compounds, and dihydroxy epoxides. For B*a*P, more than a dozen metabolites are known and they show a range of tumorigenicities (104).

Conversion of B*a*P in an inhaled MSS particle to a particular metabolite cannot be a simple process. The more than 500 PAHs in cigarette MSS range from bicyclic to decacyclic structures. In a variety of chemical reactions, the rate of reaction decreases as the molecular weight (number of rings) of the PAH increases. That is, with stoichiometric levels of the PAH and the reactant, bicyclic PAHs react faster than tricyclic PAHs which in turn react faster than tetracyclic PAHs, etc.

Diol, epoxide, and/or diol-epoxide metabolites structurally similar to those described for B*a*P have been reported for many PAHs, e.g., naphthalene, anthracene, phenanthrene, B*a*A, benzo[*c*]phenanthrene, pyrene, chrysene, DBA, benzo[*b*]triphenylene, and DMBA (104). All of these and structurally similar PAHs have been reported as cigarette MSS components (108).

In a situation, such as the formation of metabolites, where an equimolar mixture of bicyclic through hexacyclic PAHs is present, a pentacyclic aromatic hydrocarbon such as B*a*P will form little of its metabolite(s) compared to the levels formed by a more reactive bicyclic or tricyclic aromatic hydrocarbon. Numerous *in vitro* studies have demonstrated that inclusion of equimolar quantities of lower molecular weight PAHs, such as phenanthrene or anthracene, inhibits the hydroxylation-epoxidation of B*a*P in hepatic microsomes (109). However, PAH data from HOFFMANN and WYNDER (110) and RODGMAN and COOK (111) indicate the PAH classes (bicyclic, tricyclic, etc.) in MSS are present at significantly higher molar levels than the pentacyclic PAHs which include B*a*P and DBA.

In an *in vitro* study, the nontumorigenic PAHs pyrene and fluoranthene significantly inhibited the binding of a tumorigenic PAH to calf thymus DNA (enzyme source = mouse skin homogenate) (SLAGA and BOUTWELL, 112; SLAGA *et al*., 113). The *in vitro* inhibition of the hydroxylation reaction is paralleled by a reduction of *in vivo* tumorigenicity.

Because of their vapor pressure properties, tumorigenic PAHs (B*a*P, DBA, etc.) and aza-arenes are present primarily in the MSS particulate phase. Similarly, many of the reported anticarcinogens or inhibitors occur in the MSS particulate phase (9,42,114), e.g., high molecular weight alkanes (115), β-sitosterol and cholesterol (115), α-tocopherol (116), indole (117), indole-3-acetonitrile (118), duvatrienediols (119), and PAHs (anthracene, phenanthrene, pyrene, fluoranthene, B*e*P) [see (9)].

Despite the fact that the anticarcinogenicity of certain components of tobacco (120) and tobacco smoke (121,122)

and of tobacco smoke itself (121) has been known for over four decades, most discussions are directed at them as toxicants. Seldom is any significant discussion directed at smoke components known to possess anticarcinogenic properties. In a brief 1964 review of the possibility of anticarcinogenic agents in tobacco smoke, WYNDER and HOFFMANN [see pages 296, 330 in (123)] discussed the findings of STEINER and FALK (103) and KOTIN and FALK (124) in their studies with potent and weakly tumorigenic PAHs in the subcutaneous injection bioassay as well as their own findings in the mouse skin-painting bioassay (125,126). Ignored was the discussion by KOTIN and FALK (124) on the anticarcinogenicity vs. B*a*P or vs. DBA of nine PAHs (anthracene, benzo[*a*]fluorene, B*a*A, chrysene, pyrene, B*e*P, benzo[*k*]fluoranthene, benzo[*ghi*]fluoranthene, perylene), two aza-arenes (benzo[*a*]carbazole, benz[*c*]acridine), and 2-naphthol. All but the two aza-arenes had been identified in cigarette MSS prior to their 1964 review. Subsequently, the aza-arenes noted were identified as MSS components (127,128).

Earlier,WYNDER and HOFFMANN (129) had reported on MSS components that inhibited the action of a "tumorigen" invariably listed as significant. The finding was an outgrowth of their investigation of the effect of organic solvent extraction of tobacco on the PAH content of MSS. Cigarettes fabricated from the extracted tobacco yielded lower quantities of B*a*P and DBA in MSS (34,130). Skin-painting bioassays with MS CSCs from the control and extracted tobaccos gave a lower percentage of tumor-bearing animals (% TBA) in the group treated with the extracted tobacco CSC. However, the decrease in % TBA was considerably less than the percent decrease in the level of tumorigenic PAHs in the CSC (131). One explanation for the difference was that the solvent extracted almost all the alkanes from the tobacco. Thus, the alkanes were absent from the MSS from extracted-tobacco cigarettes. This fraction (constituting about 3% of MS CSC) was reported to significantly inhibit the tumorigenicity of B*a*P (126,129,132).

Mouse skin-painting studies with B*a*P and the alkanes *n*-hentriacontane and *n*-pentatriacontane showed they significantly inhibit B*a*P tumorigenicity (126,129,132). The MSS of a cigarette delivering 20 mg of CSC contains about 0.6 mg (600000 ng) of the alkane fraction and 10 ng of B*a*P, an alkane fraction:B*a*P ratio of 60000:1, far in excess of the ratios that produced significant inhibition of B*a*P tumorigenicity (WYNDER and HOFFMANN, 57,123,129).

WYNDER and HOFFMANN [see pp. 245–247, 628 in (57)] again discussed anticarcinogenic components of tobacco smoke:

> Any discussion of as complex a carcinogen as tobacco smoke should at least mention the existence of anticarcinogens. These are substances that reduce or "neutralize" the effect of a carcinogen by reacting with the carcinogen or a carcinogenic metabolite, thereby deactivating it, or by competing for reaction with cell constituents, or by interfering with the resorption of a carcinogen . . .
>
> The existence of anticarcinogens, however, must be considered in evaluating any complex mixture such as tobacco smoke condensate . . .
>
> An explanation of the tumorigenic activity of tobacco smoke condensate in terms of single constituents is made more difficult by the presence of substances that may act as anticarcinogens and/or absorption retarders, especially for tumorigenic agents. It is known that structurally related noncarcinogenic hydrocarbons can inhibit the effect of carcinogenic hydrocarbons . . .
>
> Several investigators have noticed some inhibition of tumor growth by tobacco smoke condensate . . . [including] HOFFMAN and GRIFFIN [122] . . . FALK *et al*. [120] . . . [and] HOMBURGER and TREGIER [*sic*] [133] . . . it should not come as a surprise that a material which has been proved to be carcinogenic may also interfere with tumor development, if not with tumor initiation . . .

They also noted [see pp. 370–371, 628–629 in (57)]:

> An explanation of the tumorigenic activity of tobacco smoke condensate in terms of single constituents is made more difficult by the presence of substances that may act as anticarcinogens and/or absorption retarders, especially for tumorigenic agents. It is known that structurally related noncarcinogenic hydrocarbons can inhibit the effect of carcinogenic hydrocarbons. The same interrelationship may apply to tumor-promoting and nontumor-promoting phenols.

Numerous compounds demonstrated in various bioassays to be highly effective anticarcinogens against many MSS toxicants have been identified in tobacco smoke at per cigarette delivery levels far in excess of those of the alleged tumorigens. Seldom have these anticarcinogenic MSS components been discussed in the numerous reviews of the biological properties of MSS. Even though some of the earliest data on MSS components, e.g., the alkanes, that inhibit B*a*P tumorigenicity in the skin-painting bioassay were provided by WYNDER and HOFFMANN [see pages 370–371, 628–629 (57), (126)], they more often preferred to discuss alkanes as major precursors of tumorigenic PAHs in MSS [(see pp. 496–501 in (57), (110), (126), (134)] rather than inhibitors of B*a*P tumorigenicity. MSS components reported to possess significant inhibitory or anticarcinogenic action against various tumorigenic PAHs and NNAs in MSS have been cataloged (9,42,114).

Those opposed to cigarette smoking view the complex mixture MSS differently from other complex mixtures such as raw or cooked foods, gasoline and diesel engine exhausts, factory effluents, etc. [see (135,136)]. Most are reluctant to accept the premise that a nontumorigenic component will offset the tumorigenicity of a tumorigen in animals treated with the complex mixtures CSC, MSS, SSS, or environmental tobacco smoke (ETS) containing the two (137).

Other MSS components may have also influenced the mouse skin-painting results obtained with control tobacco and extracted tobacco CSCs. Hexane extraction of tobacco not only removes alkane inhibitors thus making impossible their transfer to MSS but also removes substantial amounts of β-sitosterol (138), α-tocopherol (116,139), indole (117), duvatrienediols (119,140), and *D*-limonene (141,142), thus eliminating or drastically reducing their transfer to MSS during smoking. Subsequently, it was demonstrated that: a) These smoke components are present by transfer from tobacco to MSS during smoking and to SSS during smolder between puffs or they are generated during smoking. b) The compounds listed are anticarcinogenic vs several of the listed tumorigens, e.g., PAHs, NNAs, ethyl carbamate. However, in the 1950s, neither the identity of several of these tobacco or smoke components nor their anticarcinogenicity was known.

Comparison of identified MSS components (4) with lists of compounds (135,143) that possess inhibitory or anti-

carcinogenic action in tumorigenesis studies reveals not only that MSS contains many anticarcinogens but also that their MSS levels usually exceed those of the components listed as significant tumorigens. Previously we discussed a few inhibitory and anticarcinogenic MSS components, but they represent a small sample of the MSS components reported to exhibit such properties. From the review by SLAGA and DIGIOVANNI (135) and other reports (143), we compiled a list of MSS components reported to counteract the tumorigenicity of MSS toxicants (Table 11).

From the per cigarette MSS deliveries (Table 11), it may be calculated that the tumorigenic PAHs listed contribute from 4 to 10 μg/g of MS CSC. Nontumorigenic PAHs (naphthalene, anthracene, pyrene, phenanthrene, fluoranthene, benzo[*e*]pyrene, benzo[*b*]triphenylene) total 90 to 180 μg/g of CSC. The anticarcinogenic effect of nontumorigenic PAHs and weakly tumorigenic or nontumorigenic aza-arenes vs. carcinogenic PAHs has been known since the 1940s (103,135).

An interesting aspect of Table 11 is that it includes the dioxins as antitumorigens. SLAGA and DIGIOVANNI (135) summarized the studies in which dioxins were shown to interfere with the enzyme pathways responsible for tumorigenesis of several of the most potent PAHs. The dioxins were not listed as MSS toxicants in previous tabulations similar to Table 11 (9,42,114). In fact, only one toxicant list issued since 1990 (33) has included the dioxins even though their presence in MSS was known in 1980 (60). Is the omission of such MSS toxicants related in any way to the fact that dioxins are significant antitumorigens vs. some of the most potent mouse-skin tumorigenic PAHs present in MSS? The 1964 Advisory Committee in Chapter 6 of its 1964 Report mentions that 27 nontumorigenic PAHs had been identified in MSS, but none by name [see Chapt. 6, p. 55 in (6)]. Was the omission of their identities related to the fact that several were known to be antitumorigenic to several potent mouse-skin tumorigens such as B*a*P?

## 17 ANTIMUTAGENS IN CIGARETTE MSS

In a review of antimutagens and inhibitors of mutagenesis, RAMEL *et al.* (162) discussed the many antimutagens found naturally occurring in plants. They did not discuss tobacco but did discuss the natural occurrence of the following antimutagens: α-tocopherol, 2*H*-1-benzopyran-2-one, 7-hydroxy-2*H*-1-benzopyran-2-one, and 3-phenyl-2-propenal. All four have been identified in tobacco; all but 7-hydroxy-2*H*-1-benzopyran-2-one have been found in MSS.

LEE and REED (163) investigated the antimutagenicity of nicotine vs. *N*-nitrosodimethylamine (NDMA) and nicotine vs. B*a*P in the Ames test (*Salmonella typhimurium* TA 100). They observed that nicotine inhibits the mutagenicity of NDMA but not of B*a*P. Although the mechanism of this antimutagenicity was not elucidated, the more recent report by MURPHY and HEILBRUN (164) on the inhibition of NNN metabolism by nicotine suggests nicotine inhibition of NNA activation may be involved. LEE *et al.* (157) repeated the earlier experiment and not only confirmed the antimutagenic effect of nicotine on NDMA but also the similar activity of nornicotine and cotinine. Recently BROWN *et al.* (158) reported the antimutagenicity of nicotine and cotinine vs. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). LEE *et al.* (165) reported that CSC inhibits the mutagenic activity of several *N*-heterocyclic amines when tested in the Ames assay with *Salmonella typhimurium* TA 98 in the presence of the S-9 activation system. The mutagenic *N*-heterocyclic amines tested included Glu-P-1, Glu-P-2, Trp-P-1, Trp-P-2, IQ, and MeIQ. These compounds are among the most potent mutagens known (166–169). Several have also been reported to be tumorigenic in mammalian bioassays (170). In one of the first demonstrations of antimutagens in tobacco smoke, LEE *et al*. (165) reported that 50 to 100 μg of CSC per plate suppresses the mutagenic activity of these compounds by as much as 80%. Enzymatic studies indicate that CSC is a potent inhibitor of cytochrome P-450 dependent monooxygenase. Therefore, it appears that CSC exerts its antimutagenicity by inhibiting the P-450 system. LEE *et al*. (165) subsequently reported that fractionation of CSC yields fractions that show low mutagenicity themselves but are significantly antimutagenic.

Only a few of the listed MSS tumorigens have ever been tested for tumorigenicity to lung tissue by exposure of animals via inhalation. The results with all but one of the four MSS components (B*a*P, *N*-nitrosodimethylamine, *N*-nitrosodiethylamine, polonium-210), tested via inhalation at dose levels substantially exceeding those in MSS, were rated "equivocal" (171). Only polonium-210, administered via inhalation at massive dose levels to rats, produced squamous cell carcinoma, the lung tumor type similar to that associated statistically with cigarette smoking. However, the SURGEON GENERAL (43,172) and HOFFMANN and HECHT (7) discounted the effect of polonium-210 in MSS in lung-cancer causation in active smokers. From the type of evidence available presently, it is doubtful that many of the toxicants should be included in the various lists. Examination of data and reports on the tobacco smoke components present in one or more of the many lists sustains the premise that it is inappropriate to use such lists as evidence of any relationship between exposure to MSS and lung cancer induction in smokers or exposure to ETS and lung cancer induction in nonsmokers.

Several specific components could and should be excluded from the toxicant lists for reasons other than the failure to induce lung tumors via inhalation. a) By the early 1960s, dibenzo[*a,l*]pyrene had been reported in MSS by several groups [see account in (34)]. For its identification, the investigators relied on a published UV spectrum purportedly that of synthetic dibenzo[*a,l*]pyrene (dibenzo[*def,p*]chrysene). However, in 1966 it was demonstrated that the published spectrum was that of an isomer, dibenz[*a,e*]aceanthrylene (dibenzo[*a,e*]fluoranthene) (173). b) Previously we noted the failure by many research groups between 1963 and 2000 to confirm the presence in MSS of the tumorigenic aza-arenes reported by VAN DUUREN *et al*. (55). Dibenz[*a,j*]acridine was reported recently by RUSTEMEIER *et al*. (21). c) The precursors of arsenic and NDELA in MSS have been banned from US tobacco agronomy since 1952 and 1981, respectively.

**Table 11. Inhibitors, anticarcinogens, and antimutagens in tobacco smoke [a]**

| Component | CAS no. | Approx. delivery µg/g MS CSC | Effective against | AT, AM [b] | References [c] |
|---|---|---|---|---|---|
| | | *Hydrocarbons, aliphatic* | | | |
| Saturated aliphatic hydrocarbons [d] | | 30000 | B*a*P | AT | Wynder and Hoffmann (126) |
| e.g., $C_{31}H_{64}$ | 630-04-6 | [2500] [e] | | | |
| $C_{35}H_{72}$ | 630-07-9 | | | | |
| *D*-Limonene | 5989-27-5 | 15–50 | NNK | AT | Wattenberg and Coccia (144) |
| | | | DB[*a,i*]P | AT | Homburger *et al.* (145) |
| | | *Hydrocarbons, aromatic* | | | |
| Benzene | 71-43-2 | 480–1900 | B*a*P, DBA | AT | Crabtree (100) |
| Naphthalene | 91-20-3 | 80–160 | B*a*P, DBA | AT | Crabtree (100) |
| Anthracene | 120-12-7 | 4–7 | B*a*P, DBA | AT | Crabtree (100) |
| Phenanthrene | 85-01-8 | 2–4 | DMBA | AT | DiGiovanni *et al.* (107) [c] |
| Fluoranthene | 206-44-0 | 3–4 | DMBA | AT | DiGiovanni *et al.* (107) [c]; Slaga *et al.* (106) [c] |
| Pyrene | 129-00-0 | 3–4 | DMBA | AT | DiGiovanni *et al.* (107) [c]; Slaga *et al.* (106) [c] |
| Benz[*a*]anthracene | 56-55-3 | 0.8–2.8 | DBA | AT | Steiner and Falk (103) |
| Benzo[*e*]pyrene | 192-97-2 | 0.2 | DMBA | AT | DiGiovanni *et al.* (107) [c]; Slaga *et al.* (106) [c] |
| Benzo[*b*]triphenylene[f] | 215-58-7 | 0.05 | MC, DBA, DMBA | AT | Slaga and Boutwell (112) [c]; Slaga *et al.* (106) [c] |
| | | *Alcohols* | | | |
| Ethanol | 64-17-5 | | NNN | AT | Waddell and Marlowe [c] (146) |
| | | | NNN | AM | Farinati *et al*. (147) |
| 1-Butanol | 71-36-3 | | NNN | AT | Waddell and Marlowe [f] (146) |
| 2-Propanol, 2-methyl- {*tert*-butanol} | 75-65-0 | | NNN | AT | Waddell and Marlowe [f] (146) |
| α-4,8,13-Cyclodecatriene-1,3-diol, 1,5,9-trimethyl-12- (1-methylethyl)- {α-4,8,13-duvane-1,3-diol} | 57605-80-8 | 8–20 | DMBA | AT | Saito *et al.* [c] (140) |
| β-4,8,13-Cyclodecatriene-1,3-diol, 1,5,9-trimethyl-12- (1-methylethyl)- {β-4,8,13-duvane-1,3-diol} | 57605-81-9 | 12–25 | DMBA | AT | Saito *et al.* [c] (140) |
| β-Sitosterol | 83-46-5 | 400–550 | NNA<br>PAH | AT | Wattenberg [c] (148)<br>Yasukawa *et al.* [c] |
| Cholesterol | 57-88-5 | 120–240 | NNA | AT | Cohen *et al.* [c] |
| | | *Acids* | | | |
| Acids, long-chained aliphatic | | | NNA | AM | Takeda *et al*. (149) |
| e.g., $C_{16}H_{32}O_2$ | 57-10-3 | | | | |
| $C_{18}H_{36}O_2$ | 57-11-4 | | | | |
| Benzoic acid, 3,4,5-trihydroxy- {gallic acid} | 149-91-7 | | NNA | AT | Mirvish *et al.* [c] |
| 1-Propene-1,2,3-tricarboxylic acid {aconitic acid} | 499-12-7 | | B*a*P | AT | Kallistratos [c]; Kallistratos and Fasske [c] |
| 2-Propenoic acid, 3-(3,4-dihydroxyphenyl)- {cinnamic acid, 3,4-dihydroxy-} {caffeic acid} | 331-39-5 | | B*a*P | AT | Wattenberg *et al.* [c] |
| 2-Propenoic acid, 3-(3-hydroxy-4-methoxyphenyl)- {cinnamic acid, 3-hydroxy-4-methoxy-} {ferulic acid} | 537-73-5 | | B*a*P | AT | Wattenberg (148) |
| 2-Propenoic acid, 3-(2-hydroxyphenyl)- {cinnamic acid, 2-hydroxy-} | 614-60-8 | | B*a*P | AT | Wattenberg *et al.* [f] |
| 2-Propenoic, 3-phenyl- {cinnamic acid} | 621-82-9 | | NPYR, NNN | AT | Chung *et al.* (150,151) |
| | | *Phenols* | | | |
| Phenol | 108-95-2 | 1000–7000 | B*a*P | AT | Van Duuren *et al.* (152) |
| | | | NNN, NPYR | | Chung *et al.* (150,151) |
| Phenol, 4-methoxy- | 150-76-5 | | B*a*P | AT | Wattenberg *et al.* [f] |
| α-Tocopherol {vitamin E} | 59-02-9 | 400–600 | MC, DMBA, DB[*a,i*]P, 1,2-DMH | AT | Shamberger [c]; Shklar [c]; Slaga and Bracken [c]; Viaje *et al.* [c]; Weerapradist and Shklar [c] |
| | | | NNA | AT | Thompson (153) |
| | | | CSC | AM | Rosin [c] |
| 2*H*-1-Benzopyran-2-one, 6,7-dihydroxy- {esculetin} | 305-01-1 | | NNK | AT | Teel and Castonguay (154) |

**Table 11 (*cont.*)** [a]

| Component | CAS no. | Approx. delivery µg/g MS CSC | Effective against | AT, AM [b] | References [c] |
|---|---|---|---|---|---|
| | | | N-*Containing components* | | |
| Indole | 120-72-9 | 400–600 | NNA | AT | Matsumoto *et al.* [c] |
| | | | NNN, NPYR | | Chung *et al.* (150,151) |
| | | | NNK | | Chung *et al*. (155) |
| Indole-3-acetonitrile | 771-51-7 | | BaP | AT | Kovacs and Somogyi [c] |
| 1*H*-Purine-2,6-dione, 3,7-dihydro-3,7-dimethyl- {theobromine} | 83-67-0 | | EC | AT | Nomura [c] |
| 1*H*-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl- {caffeine} | 58-08-2 | | EC, DMB, NNA | AT | Nomura [c]; Perchellet and Boutwell [c]; Mirvish *et al.* [c] |
| Nicotine | 54-11-5 | | NNK | AT | Schüller *et al*. (156) |
| | | | NDMA | AM | Lee *et al.* (157) |
| | | | NNAL | AM | Brown *et al*. (158) |
| Nornicotine | 494-97-3 | | NDMA | AM | Lee *et al.* (157) |
| | | | NNAL | AM | Brown *et al*. (158) |
| Cotinine | 486-56-6 | | NDMA | AM | Lee *et al.* (157) |
| | | | NNAL | AM | Brown *et al*. (158) |
| | | | *Miscellaneous components* | | |
| 2*H*-Benzopyran-2-one {coumarin} | 91-64-5 | | BaP, DMBA | AT | Wattenberg *et al.* [c] |
| 3*H*-2-Furanone, dihydro-5-methyl- {α-angelica lactone} | 108-29-2 | | BaP | AT | Wattenberg *et al.* [c] |
| Benzoic acid, 3,4,5-trihydroxy-, propyl ester [d] {propyl gallate} | 121-79-4 | | NNK | AT | Lo and Stich [c]; Teel and Castonguay (154) |
| Dioxin | | | DMBA, MC, BaP, 7-MBA, 12-MBA, 5-MeC, DBA | AT | Berry *et al*. (159); Cohen *et al*. (160); DiGiovanni *et al*. (161) |
| Carbon disulfide | 75-15-0 | | 1,2-DMH | AT | Wattenberg and Fiala [c] |
| Maleic anhydride | 108-31-6 | | PAH, DMBA | AT | Klein [c]; Slaga *et al.* [c] |
| Selenium | 7782-49-2 | | DMBA | AT | Shamberger [c] |
| | | | NNA | AT | Thompson (153) |
| Cysteine | 52-90-4 | | NDMA | AT | Lo and Stich [c] |

[a] *Abbreviations*: BaP = benzo[*a*]pyrene; DBA = dibenz[*a,h*]anthracene; DB[*a,i*]P = dibenzo[*a,i*]pyrene, = benzo[*rst*]pentaphene; DMBA = 7,12-dimethylbenz[*a*]anthracene; 1,2-DMH = 1,2-dimethylhydrazine; 7-MBA = 7-methylbenz[*a*]anthracene; 12-MBA = 12-methylbenz[*a*]anthracene; 5-MeC = 5-methylchrysene; EC = ethyl carbamate; MC = 3-methylcholanthrene, = 1,2-dihydro-3-methylbenz[*j*]aceanthrylene; NDMA = *N*-nitrosodimethylamine; NNA = *N*-nitrosamine; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNN = *N'*-nitrosonornicotine; NNK = 4-(*N*-methylnitrosamino)-1-(3-pyridinyl)-1-butanone; NPYR = *N*-nitrosopyrrolidine; PAH = polycyclic aromatic hydrocarbon.

[b] AT = test for antitumorigenicity; AM = test for antimutagenicity.

[c] Representative references to inhibition, anticarcinogenicity, and/or antimutagenicity. Details of this reference may be found in Fay *et al*. (143) and/or Rodgman (42). Additional references may be found in (42,107,135,143).

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

[e] Average weight (µg/g MS CSC) of each hydrocarbon isomer.

[f] Benzo[*b*]triphenylene was formerly known as dibenz[*a,c*]anthracene.

## 18 THE COMPENSATION ASSERTION

Because of the lack of derogatory evidence from the anti-tobacco critics about added ingredients and the gradual accumulation of evidence that the usual or increased levels of added ingredients produced no significant adverse effect on the chemical and biological properties of the MSS, criticism was shifted from the added ingredient issue to compensation, i.e., the smoker is taking more puffs, larger puffs, and more particularly, blocking the filter-tip ventilation holes of the cigarette to compensate personally for the lower "tar" and nicotine deliveries as measured in the FTC procedure. The compensation issue and discussions of it have become so massive that the issue is much too detailed to be dealt with at length in our review. Much of the issue has been put in perspective by SCHERER (174) and by BAKER and LEWIS (175).

## 19 THE RISE AND FALL OF THE MAJOR CIGARETTE MSS TOXICANTS: EXCEPTION – THE TOBACCO-SPECIFIC *N*-NITROSAMINES (TSNAs)

Since the mid-1950s various MSS toxicants, either as an individual component or a class of components, have had their moment of publicity but one by one their importance gradually faded. Chronologically, the first toxicants to become infamous were the tumorigenic PAHs with B*a*P at the pinnacle because of its potent tumorigenicity to mouse skin and its level in cigarette MSS. The chronological sequence of the rise to notoriety of the various individual and/or class of toxicants has been previously depicted [see Figure 1 in RODGMAN *et al*. (176)] but the depiction does not show when the prominence of most of them declined.

In the mouse skin-painting bioassay, neither B*a*P nor the total tumorigenic PAHs account for the observed specific tumorigenicity (177). The B*a*P content of CSC accounts for less than 2.5% and the total tumorigenic PAH content of CSC accounts for less than 3.5% of the CSC specific tumorigenicity [see Chapt. 6, p. 55 in (6), see p. 626 in (57), (178)]. Inclusion of tumorigenic aza-arenes reported by VAN DUUREN *et al.* (55) does not improve the situation. HOFFMANN and WYNDER (110) reported that doubling or tripling the level of 17 tumorigenic PAHs in CSC significantly increases the % TBA (tumor-bearing animals) whereas others reported that a 10-fold (179) or 30-fold (180) increase in the B*a*P level in CSC produces no change in the % TBA. In the early 1960s, the promoting effect of the MSS phenols on tumorigenic PAHs was advanced to explain the tumorigenic response observed in CSC-painted mice. Inclusion of this effect in the assessment accounted for about 5% of the % TBA. In addition, reports of no change in the tumorigenicity of CSC when significant amounts (75–90% ) of the phenols were removed from MSS (and the CSC) by selective filtration [see p. 626 in (57), (181)] and the inhibition of the specific tumorigenicity of B*a*P by phenol (152) diminished the alleged importance of the promoting effect of phenols.

To offset the decrease in importance of the PAHs, azaarenes, and phenols, ciliastatic components in MSS then became the in-vogue toxicants. It was asserted, based on studies with clam cilia and mammalian ciliated tissue, that certain MSS toxicants impair lung ciliary activity thus preventing removal of tumorigen-containing smoke particles from the lung [see references in KENSLER and BATTISTA (182)]. Chief MSS ciliastats are formaldehyde, acetaldehyde, acrolein, HCN, formic and acetic acids, and phenol. However, after 1968, the ciliary assertion faded with the demonstration that less than a third of the ciliastats reach the lung cilia in human smokers (183).

In the mid-1960s, several other MSS toxicants had their brief moment of infamy, e.g., $^{210}$Po, $NO_2$, CO. In their comparison of lung cancer incidence in uranium miners exposed to $^{210}$Po vs. cigarette smokers exposed to MSS $^{210}$Po, HARLEY *et al.* (77) questioned the significance of $^{210}$Po in tobacco-induced lung cancer. Concern over $NO_2$ diminished with the demonstration that over 95% of the $NO_x$ in MSS is NO, not $NO_2$, and the conversion of NO to $NO_2$ is impeded by other MSS components (95).

In the early 1960s, the formation of *N*-nitrosamines (NNAs) during tobacco smoking was suggested (184) as well as the possible presence of *N*'-nitrosonornicotine (NNN) and *N*'-nitrosoanabasine (NAB) in MSS (185). Between 1964 and the early 1970s, several volatile NNAs were identified in MSS. It was also determined that 60% to 85% of the volatile NNAs, like the phenols, are selectively filtered from MSS. The identification of several TSNAs, including NNN and NAB, then followed.

Why have TSNAs maintained their status as important MSS toxicants while the importance of other individual and/or classes of toxicants has faded? Alternate exposures are possible with other toxicant classes including NNAs other than the TSNAs but, as their classification denotes, the TSNAs are "tobacco-specific". In the detailed 1984 outline of chemical carcinogenesis edited by SEARLE (186), the only class of MSS tumorigens discussed in 22 chapters comprising nearly 1400 pages is the NNAs!

Since the early 1960s, a "less hazardous" cigarette has been defined on the basis of three criteria [see p. iii, Report No. 1 in (35); p. 372 in (123); p. 503, 531 in (57)]: 1) the per cigarette delivery of a specific toxicant has been lowered, 2) the ratio of the specific toxicant to MSS "tar" has been lowered, and 3) the specific tumorigenicity of the MSS "tar" as measured in the mouse skin-painting bioassay has been lowered.

From bioassay results of more than 330 NNAs plus knowledge of fewer than 50 specific NNAs in MSS, it is obvious that the MSS NNAs cannot meet criterion 3). Over 330 *N*-nitroso compounds variously administered to 40 different species have been reported as tumorigenic. No laboratory species is resistant to NNAs. In their summary of the results from 323 *N*-nitroso compounds bioassayed from 1956 to 1984, PREUSSMANN and STEWART (187) reported that 87% of the *N*-nitroso compounds are tumorigenic. Over 70% of the *N*-nitroso compounds studied were NNAs; the remainder was *N*-nitrosamides.

Administration of most NNAs to laboratory animals via skin painting seldom results in carcinoma induction at the application site. Generally, tumors develop at site(s) remote from the painting site and various organs may be involved. This major difference between PAH and NNA tumorigenicity led to defining NNAs as organ-specific tumorigens. Failure to produce tumors with NNAs at the painting site subsequently led to studies of NNAs administered by alternate routes [injection (subcutaneous, intravenous, intraperitoneal), *per os*, intratracheal instillation, etc.]. Administration of NNAs by inhalation was studied infrequently.

Skin-painting studies with six NNAs (*N*-nitrosobutylmethylamine, *N*-nitrosodiethylamine, NDELA, NNK, NNN) present in tobacco and/or tobacco smoke were reported by BRUNE and HENNING (188), HOFFMANN and GRAFFI (189), HERROLD (190), the IARC (38), HOFFMANN *et al.* (191), and LAVOIE *et al.* (192). Tumors developed elsewhere in the test animals but none at the painting site. In a painting study by DEUTSCH-WENZEL *et al.* (193), NNN induced a few skin tumors, but no dose-response relationship was observed over a 12.5- to 200-µg range. In the same experiment, the tumorigenic potency to skin of *N*-nitroso-*N*-methylurea was estimated to be about 4% of that of B*a*P (193). In painting studies with *N*-nitroso-*N*-alkylureas, tumors did develop at the skin-painting site, but to date, no *N*-nitroso-*N*-alkylurea has been identified in tobacco or its smoke.

## 20 THE ARTIFACTUAL FORMATION OF *N*-NITROSAMINES

In 1964, NEURATH *et al.* (194) reported *N*-nitroso-*n*-butylmethylamine and two unidentified NNAs in MSS. The next year, NEURATH *et al.* (195) discounted their reported findings because of artifactual formation of the NNAs during their collection/analytical procedure. However, with a modified analytical and collection procedure, *N*-nitrosodimethylamine (4 ng/cig) and *N*-nitrosopyrrolidine (4 ng/cig) were identified in MSS. The previously reported *N*-nitroso-*n*-butylmethylamine was found in the part of the collection system where artifactual formation was possible. The artifactual formation of NNAs during smoke generation, separation, and analysis

has been a recognized problem since the first NNA identification in MSS (196–198).

Besides identifying several volatile NNAs in burley tobacco smoke with a procedure that precluded artifactual formation, FREDRICKSON (199) demonstrated that MSS volatile NNA levels are reduced (60–85%) by a plasticized cellulose acetate filter, a finding subsequently confirmed by others (200–202). This reduction of volatile NNA levels by selective filtration resembles that observed for phenols (203,204).

Concern over phenols and their promotion effect diminished after reports of removal of significant amounts of them from MSS by selective filtration. While concern about volatile NNAs did diminish, a new NNA concern arose: one involving tobacco-specific *N*-nitrosamines (TSNAs), a class of NNAs newly identified in tobacco and tobacco smoke, namely NNN and NAB.

Artifactual formation of volatile NNAs during smoke collection and analysis was noted in the mid-1960s by NEURATH *et al*. (194) and FREDRICKSON (199). The problem was once again revisited by KRULL *et al.* (196) who proposed methodology to reduce it. The problem resurfaced several times in the next decade in the determination of both the volatile NNAs (197,198) and TSNAs in tobacco smoke (198) and preventative measures were proposed.

HOFFMANN and HECHT (7) did not acknowledge that the MSS levels listed for both volatile NNAs and TSNAs may be incorrect (and high) because of their artifactual formation during MSS (and SSS) collection for analysis (198). The US EPA (8) accepted without question the per cigarette MSS volatile NNA and TSNA data listed by HOFFMANN and HECHT (7), and these data were also cited by the SURGEON GENERAL (43).

## 21 TSNAs IN MSS: DIRECT TRANSFER FROM TOBACCO AND CONFLICTING DATA ON FORMATION DURING THE SMOKING PROCESS

Nicotine, nornicotine, anabasine, and anatabine are precursors of TSNAs in tobacco and tobacco smoke (205, 206). Both nicotine and nornicotine are considered to be NNN precursors. Since NNAs (both volatile and tobacco-specific) occur in tobacco, a part of the NNAs in cigarette MSS was reported to be due to direct transfer of NNAs from tobacco to MSS, the remainder due to formation and transport during the smoking process (206). For NNK, the transfer from tobacco to MSS ranges from 6.9% to 11.0% of the amount in the tobacco; this represents about 30% of the NNK in MSS. Similarly, about 40% of the NNN in MSS is transferred from the tobacco. According to HOFFMANN and his colleagues, the remainder of these two TSNAs in MSS is formed during the smoking process (207,208). Like the levels of the volatile NNAs in MSS, the levels of the TSNAs in MSS are proportional to the nitrate content of the tobacco filler (209). However, the premise of the pyrogenesis of NNN and NNK has been challenged by FISCHER *et al*. (210,211) who reported that these compounds occur in cigarette MSS only by transfer from the tobacco rod. CASTONGUAY, a frequent co-author with HOFFMANN and HECHT on TSNA articles, commented that NNK is transferred from tobacco to smoke during the cigarette smoking process (212). In agreement with FISCHER *et al*., RENAUD *et al*. (213) concluded from their data on MSS TSNA levels that direct tobacco-to-smoke transfer is the dominant factor explaining the presence of TSNAs in MSS. In a study of the contribution of $^{13}$C-nicotine to the $^{13}$C-NNN and $^{13}$C-NNK levels in cigarette MSS condensate, MOLDOVEANU *et al*. concluded that NNN and NNK are generated during the smoking process (214), thus contradicting the views of FISCHER *et al*., RENAUD *et al*., and CASTONGUAY. Moreover, the pyrogenesis situation is further clouded by data on the effect of tobacco nitrate on the TSNA levels in MSS (215). Analysis of MSS TSNAs indicates that NNN and NAT levels increase when nitrate is added to the tobacco but the NNK level does not.

## 22 RISK ASSESSMENTS OF TSNAs IN CIGARETTE MSS

Several investigators have assessed the risk to the smoker of long-time exposure to NNAs in cigarette MSS, particularly the TSNAs NNK and NNN. HOFFMANN and HECHT (7) discussed the effect on a cigarette smoker of inhaling the MSS from a 1986 American nonfiltered cigarette that delivered 425 ng of NNK in its MSS. This delivery can be assessed in an alternate way as recently outlined by TRICKER (32). One can calculate not only the number of packs of cigarettes which would have to be smoked per day for 40 years but also the number of years of smoking 2 packs/day to achieve the same total TSNA exposure as the lowest dose required to induce a significant incidence of lung tumors in laboratory animals.

HECHT and HOFFMANN concluded that the PAHs and NNK are the major carcinogens involved in lung cancer induction by cigarette MSS (216). The inclusion of the PAHs was remarkable in light of numerous publications from the 1960s to 1993 in which it was reported that B*a*P alone, all the tumorigenic PAHs acting additively, and the tumorigenic PAHs plus promoting phenols account for only a small percentage (<5% ) of the % TBA observed.

In Table 12, the TRICKER calculations are applied to NNK and NNN data for the MSS from the 1R4F cigarette, data from RJRT and RICKERT and WRIGHT (217). The calculations differ slightly from those by TRICKER, being applied to smokers of 1 pack/day of the 1R4F cigarette for 35 years.

A major problem with these risk assessments is the total disregard of the admonitions made in 1941 by SHEAR and LEITER (99). They wrote:

> [T]he term "carcinogenic potency" as used in [carcinogenesis] studies is not to be considered as an invariable property inherent in a compound but is merely a summary of the results of particular experiments and is valid only for animals of the species, strain, sex, age, diet, etc., of the particular animal employed, as well as for the dose, menstruum, mode and site of application, etc., of the compound in question . . . Conclusions regarding the potency of any given compounds should therefore be interpreted in the light of the data upon which they are based.

These admonitions were considered sufficiently meaningful that HARTWELL cited them in the Introduction to his USPHS compendium on compounds tested for tumorigenicity (101).

**Table 12. Extrapolation of rodent bioassay results to a human smoker of cigarette 1R4F**

| TSNA | Laboratory animal | Lowest total dose (mg/kg body weight) [a] | Comparison to man [b] | | Comparison to man [c] | |
|---|---|---|---|---|---|---|
| | | | Packs/day for 35 years | Years of smoking 1 pack/day | Packs/day for 35 years | Years of smoking 1 pack/day |
| NNK | F344 rat | 70.5 (buccal) (218) | 199 | 6980 | 221 | 7750 |
| | F344 rat | 35.2 (p.o.) (219) | 99 | 3480 | 110 | 3870 |
| | F344 rat | 6.0 (s.c.) (220) | 17 | 594 | 19 | 659 |
| | A/J mouse | 364 (p.o.) (221) | 1028 | 36000 | 1141 | 40000 |
| | A/J mouse | 20.8 (i.p.) (222) | 59 | 2060 | 65 | 2290 |
| | SG hamster | 9.0 (s.c.) (223) | 25 | 891 | 28 | 989 |
| NNN | F344 rat | 531 (s.c.) (224) | 1264 | 44200 | 1362 | 47800 |
| | A/J mouse | 2153 (i.p.) (225) | 5126 | 179000 | 5520 | 194000 |

[a] Lowest total dose required to induce a significant incidence of lung tumors.
[b] Hypothetical total human experience of a 1 pack/day smoker for 35 years = 0.354 mg NNK and 0.42 mg NNN. These are derived from the RJRT per cigarette data for Cigarette 1R4F; NNK (97 ng), NNN (115 ng).
[c] Hypothetical total human experience of a 1 pack/day smoker for 35 years = 0.319 mg NNK and 0.39 mg NNN. These are derived from the Rickert and Wright per cigarette data (217) for Cigarette 1R4F; NNK (87 ng), NNN (107 ng).

In over 60 years, nothing has been discovered that renders these words invalid! Thus, it is inappropriate to extrapolate findings from a fed or injected or skin-painted compound administered individually to laboratory animals either neat or in solution to the effect of that compound as a component of an extremely complex mixture such as the cigarette MSS aerosol encountered by inhalation. This sentiment was expressed over two decades ago by GORI (226):

> [I]t would be unrealistic to assess the biologic effect of any smoke component or additive as an independent entity, outside of the interactions that occur in smoke.

Another problem with the induction of lung tumors in laboratory animals is the omission of the fact that most lung tumors developed by mice are adenomas. Known since the 1950s is the fact that certain mouse strains are inbred to be susceptible to adenoma development, e.g., 90% of untreated Strain A mice develop and die from adenomas (227). Administration of a tumorigen does not usually alter the % adenoma-bearing animals but may shorten the time of adenoma appearance.

## 23 TECHNOLOGIES TO CONTROL MSS TOXICANT LEVELS

Previously (Section 19), we outlined the three criteria used to define a "safer" or "less hazardous" cigarette, i.e., 1) the per cigarette delivery of a specific toxicant has been lowered, 2) the ratio of the specific toxicant to MSS "tar" has been lowered, and 3) the specific tumorigenicity of the MSS "tar" as measured in the mouse skin-painting bioassay has been lowered.

Significantly, the elimination of the first criterion as a complete definition per se of a "safer" or "less hazardous" cigarette and the requirement that all three criteria in the definition be met arose because personnel at various research institutions wished to avoid the appearance of endorsing low-"tar" cigarettes.

Since the early 1950s, it might appear that the cigarette design efforts of the Tobacco Industry R&D personnel were primarily directed to meeting these criteria. However, the R&D personnel in general were troubled by the overall definition and viewed two of the criteria as seriously flawed. Criticisms of these criteria were not limited to Tobacco Industry scientists but were also expressed by scientists with anti-tobacco smoking views.

Various members of the anti-tobacco smoking group expressed conflicting opinions on the first criterion. Some interpreted the experimental evidence of lower % tumor-bearing animals in mice treated with reduced levels of "tar" (equivalent to reduced cigarette delivery) as an indication that a lower-"tar" delivery cigarette is "safer" or "less hazardous" than a higher-"tar" delivery cigarette. Others held the view that the biological response resulted from a dose-response factor.

The second criterion for a "safer" or "less hazardous" cigarette is paradoxical. On the one hand, some of its proponents recommended the reduction of the levels of specific components in MSS supposedly responsible for the observed tumorigenicity of particulate matter to mouse skin. However, on the other hand, other proponents of this criterion admitted either an inability to explain the observed biological effect on the basis of the levels of these components in the particulate matter or they accepted (and still accept) the lack of an association between the observed biological effect and chemical composition!

The third criterion suffers from several problems: It ignores the findings that a) inhalation studies with laboratory animals exposed to cigarette MSS have consistently given inconclusive (negative) results with regard to carcinoma induction, b) mouse skin-painting bioassays with cigarette smoke particulate matter do not measure smoke components reported to be tumorigens in other systems, e.g., NNAs, and c) skin-painting and Ames test data with cigarette MSSs produced under certain conditions are widely divergent. Recently, some departure from the third criterion has occurred with the increased usage of various cytotoxicity tests.

## 24 CIGARETTE DESIGN TECHNOLOGIES STUDIED AND REJECTED

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

Solvent extraction of tobacco, while removing lipophilic PAH precursors, increases the levels of nitrate and the biopolymers lignin, cellulose, pectins, and starch in the extracted tobacco by a factor of 8% to 12%. Increasing lignin and carbohydrates levels, known phenols precursors, increases delivery of MSS phenols classified as promoting toxicants. However, assertions that phenols are promoters were offset by reports that a) almost complete removal of phenols from cigarette MSS by selective filtration produces little change in the specific tumorigenicity of the CSC to mouse skin (110,204,228–230) and b) phenol, supposedly the most potent promoter of PAH tumorigenicity, inhibits B*a*P tumorigenicity to mouse skin (152). Increasing the tobacco carbohydrates level also increases the MSS levels of several aldehydes, ketones, and acids defined as *in vitro* ciliastats. Here again, their importance as contributors to respiratory tract cancer induction was substantially diminished when studies in smokers revealed that a large proportion of most *in vitro* ciliastats never reach the ciliated areas of the lung (183).

While the presence of NNAs in MSS had been predicted in 1962 (231), their presence in cigarette MSS and the positive relationship between tobacco nitrate level and the NNA levels in tobacco and smoke were not defined until later. Thus, organic solvent extraction of tobacco might be categorized as beneficial because of reduction of mouse-skin tumorigen levels (PAHs) in the MSS but categorized as detrimental because of the increase in MSS levels of other toxicants, the supposed promoters (phenols), cocarcinogens (phenols), ciliastats (vapor-phase aldehydes, ketones, acids), and organ-specific tumorigens (NNAs).

Unknown in the 1950s was the fact that the extraction also removed tobacco components subsequently reported to be inhibitors (alkanes) of B*a*P tumorigenicity (126,203) or anticarcinogenic (α-tocopherol, duvanediols) against the potent tumorigens B*a*P and DBA (9). Absence or significant depletion of these inhibitors and anticarcinogens from the extracted tobacco was accompanied by substantial reduction of their delivery levels in cigarette MSS. As a result of these and other factors, the process of solvent extraction of tobacco as a cigarette design technology was abandoned. Investigators outside of the Tobacco Industry classified the process as "impractical both technically and economically" (232) and "of academic interest only" (233), a sentiment echoed by the US SURGEON GENERAL [see p. 114, Table 26 in (43)].

The second method studied to reduce PAH levels in cigarette MSS was the use of "catalysts" to modify the combustion process during smoking. The most effective were nitrates that during smoking generated NO that interfered with the free radical mechanism involved in PAH formation. For several years prior to identification of NNAs in tobacco and tobacco smoke, increasing the blend nitrate level was examined as a means to lower the tumorigenic PAH levels in MSS and the specific tumorigenicity of the MS CSC to mouse skin. Nitrate addition lowered several classes of MSS toxicants, the PAHs (57,110,123,230,234,235) and phenols (230,236). Because tobacco stems were usually high in nitrate, inclusion of stem-based reconstituted tobacco sheet (RTS) in the blend was proposed and studied (35,237–239). Another way to increase the nitrate level of the blend was to incorporate high-nitrate tobaccos, a technology examined extensively (200,240–242). Because of the demonstration of the relationship between tobacco nitrate level and the NNA levels in MSS (209,242,243), the original proposals were superseded by new ones: *Incorporate low-nitrate tobaccos in the blend and/or remove the nitrates from the tobacco* (237).

A third method proposed to reduce the level of PAHs in cigarette MSS was the inclusion of a compound in the filter tip that would complex with the PAHs and their nitrogen analogs (244). The aerosol nature of cigarette MSS precludes the success of this approach.

As mentioned previously, research to reduce the levels of individual MSS components or classes of MSS components was replaced by research to reduce MSS components, both vapor- and particulate-phase components, uniformly across the board as much as possible. Such an approach had been voiced by numerous authorities both within and outside of the Tobacco Industry, e.g., DALHAMN's quote of RYLANDER's 1967 comment (245).

Table 13 summarizes the effect of these technologies, eventually rejected, on cigarette MSS properties.

## 25 CIGARETTE DESIGN TECHNOLOGIES STUDIED AND INCORPORATED INTO COMMERCIAL PRODUCTS

In the design of a "less hazardous" cigarette, many approaches have been investigated. Table 14 summarizes the technologies studied by Tobacco Industry and non-Industry investigators, a list eventually reduced to the eight technologies in Table 15.

Their chronological impact on sales-weighted cigarette MSS "tar" and nicotine deliveries has been noted frequently [cf. Figure 3 in RODGMAN (34)]. By the early 1960s, several cigarette design technologies developed by the Tobacco Industry and used in commercial products were categorized as significant in their contribution to the "less hazardous" cigarette. Ultimately, the initial four design technologies (tobacco blend, effective and efficient filtration, RTS, air dilution via cigarette paper porosity) were increased to eight.

Their significance was recognized in "less hazardous" cigarette design by the NCI[1] and the US Surgeon General.

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

**Table 13. Effect of discarded technologies on properties of cigarette mainstream smoke**

| Smoke component and/or property | Phase particulate (PP) or vapor (VP) | Technology | | |
|---|---|---|---|---|
| | | Solvent extraction | Combustion catalyst [a] | Filter-tip additive [b] |
| *FTC "tar"* | PP | ⇓ [c] | ⇓ | ⇓ [d] |
| Specific tumorigenicity | PP | ⇓ [e] | ⇓ | — |
| Specific mutagenicity | PP | ⇑ | ⇑ | — |
| PAHs | PP | ⇓ [c] | ⇓ | ⇓ [d] |
| Phenols | PP & VP | ⇑ | ⇓ | — |
| *N*-Nitrosamines | PP & VP | ⇑ | ⇑ | — |
| *Inhibitors/anticarcinogens* | | | | |
| Long-chain alkanes | PP | ⇓ | — | ⇓ |
| PAHs [f] | PP | ⇓ | — | ⇓ |
| Duvanediols | PP | ⇓ | ⇓ | ⇓ |
| α-Tocopherol | PP | ⇓ | ⇓ | ⇓ |

[a] Nitrates were shown to be the most effective combustion catalysts.
[b] An additive that forms stable complexes with PAHs and aza-arenes, e.g., chloranil, 2,4,7-trinitrofluorenone.
[c] ⇓ indicates property or component may be lowered by appropriate choice of blend. ⇑ indicates property or component may be increased by use of a particular technology.
[d] Per cigarette deliveries of FTC "tar", PAHs (both tumorigenic and anticarcinogenic) reduced by same percentage due to increase in pressure drop across the additive-treated filter tip.
[e] Decrease in % TBA was much less than % decrease in MSS levels of tumorigenic PAHs such as B*a*P.
[f] Includes naphthalene, anthracene, phenanthrene, fluoranthene, pyrene, benzo[*e*]pyrene, benzo[*b*]triphenylene, benz[*a*]anthracene.

**Table 14. Alteration of cigarette mainstream smoke yield, composition, and biological activity: Methods studied**

| Cigarette design technology | |
|---|---|
| *Tobacco selection* | *Tobacco additives* |
| Type | Combustion modifiers |
| Stalk position | Casing materials and humectants |
| Nitrate content | Flavorants |
| Nicotine content | Pesticides, agricultural chemicals |
| Other components | |
| *Tobacco treatment* | *Cigarette paper* |
| Curing | Porosity (air dilution) |
| Grading | Additives |
| Fermentation | Coatings |
| Extraction | |
| Denicotinization | *Filtration* |
| Ammoniation | Efficiency/selectivity |
| Expansion (laminae and/or stems) | Additives |
| | Material (cellulose acetate, paper) |
| | Material (charcoal) |
| *Blending* | |
| *Tobacco cut width* | *Air dilution (perforated filter tips)* |
| *Amount of tobacco* | *Diluents (substitutes*) |
| Cigarette dimensions | Cytrel® |
| Tobacco weight | NSM® (New Smoking Material) |
| RTS (nonpaper) | Expanded grains |
| RTS (paper) | Carbon/carbonized filler |
| Homogenized leaf | SSM® (Sutton Smoking Material) |
| Stem inclusion | Other plants (lettuce, peanut hulls, etc.) |
| Expanded laminae | |
| Moisture content | |

In Table 15 are listed chronologically (1960 through 1997) some of the reports in which various authorities commended these eight design technologies.

Table 16 summarizes the effect of these technologies plus tobacco ammoniation on some of the major cigarette MSS properties. A technology that primarily influences the particulate-phase yield generally influences the MSS levels of those components defined as particulate-phase toxicants. Similarly, a technology that primarily influences the vapor-phase yield generally influences the MSS levels of those components defined as vapor-phase toxicants. From 1913 to the early 1950s, the major design technology employed was the tobacco blend. Chronologically, the rodent skin-painting bioassay became available, specific PAHs were defined as tumorigenic to laboratory animals, but prior to 1954 little was known about the composition of tobacco smoke.

While each design technology may be used to control MSS yield and composition, none is now used individually. The eight design technologies listed in Table 15 are used in concert and to different degrees, thus enabling the design of consumer acceptable cigarettes with MSS FTC "tar" deliveries ranging from 1 to 40 mg/cig and MSS nicotine deliveries ranging from 0.1 to 3.0 mg/cig.

Of course, the initial thrust of this across-the-board reduction was aimed at reducing the MSS "tar" yield because of extrapolation by WYNDER *et al*. (246) of their 1957 mouse-skin bioassay findings:

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

In his 1957 testimony during the filter-tipped cigarette hearings, WYNDER (36) reiterated his opinion that reducing "tar" exposure dose by 40% to 50% would substantially reduce lung cancer induction in smokers.

Examination of the graphical representation of the sales-weighted average "tar" yield for US commercial cigarettes

**Table 15. Cigarette design technologies recognized as contributing to less hazardous cigarettes [a,b]**

| Design technology | W&H 1960 | W&H 1964 | W&H 1965 | W&H 1966 | W&H 1967 | W&H 1969 | W&He 1976 | NCI 1976–80 | HSHW 1978 | US SG 1979 | W&H 1979 | LHHW 1980 | US SG 1981 | H&W 1986 | H&H 1997 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tobacco blend [c] | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Filter tip [d] | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Filter-tip additive [e] | — | X | X | — | X | X | X | X | X | X | X | X | X | X | X |
| RTS [f] | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Paper additive [g] | — | X | X | — | — | — | — | X | — | X | — | — | — | — | X |
| Air dilution (paper porosity) [h] | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Expanded tobacco [i] | — | — | — | — | — | — | X | X | X | X | X | X | X | X | X |
| Air dilution (filter-tip perforation) [j] | — | — | — | — | — | — | X | X | X | X | X | X | X | X | X |

[a] Technologies cited in US Surgeon General's 1979, 1981, and 1982 smoking-and-health reports [see pp. 104–114 in (43), see pp. 217–218 in (172), (253)].

[b] W&H 1960 = Wynder and Hoffmann (247); W&H 1964 = Wynder and Hoffmann (123); W&H 1965 = Wynder and Hoffmann (248); W&H 1966 = Wynder and Hoffmann (249); W&H 1967 = Wynder and Hoffmann [see p. 503 in (57)]; W&H 1969 = Wynder and Hoffmann (250); W&H 1979 = Wynder and Hoffmann (251); W&He 1976 = Wynder and Hecht (233); NCI 1976–80 = Gori (35); NCI (35); HSHW 1978 = Hoffmann *et al.* (252); US SG 1979 = USPHS [see pp. 104–114 (43)]; US SG 1981 = USPHS (253); LHHW 1980 = Lavoie *et al.* (79); H&W 1986 = Hoffmann and Wynder (254); H&H 1997 = Hoffmann and Hoffmann (255)

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

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

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

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

[g] In 1958, citrates were added to cigarette paper for more uniform combustion of the tobacco rod.

[h] In 1959, increased cigarette paper porosity was introduced as a means to lower MSS "tar" and nicotine yield.

[i] Expanded tobacco laminae were incorporated into commercial products in the late 1960s. US patents were issued in 1970 (256).

[j] A product with a perforated filter tip was introduced commercially in the US in the late 1960s.

[cf. Figure 3 in RODGMAN (34)] reveals that the 40% to 50% reduction in MSS "tar" yield considered vital by WYNDER in 1957 was achieved in the late 1960s, i.e., a reduction from 38–39 mg/cig to 19–20 mg/cig. Further examination reveals that by the early 1980s, the sales-weighted average "tar" was further reduced to about 12 mg/cig, i.e., an additional 40% reduction had been achieved. Corresponding reductions in the MSS deliveries of total PAHs in general, B*a*P in particular [see pp. 111–112 in (43), (257)], and nicotine were also achieved.

Reminiscent of the numerous lengthy review articles issued in the 1980s and early 1990s on the biological properties of NNAs, particularly TSNAs, in MSS (258,259) is the recent flood of highly repetitious articles devoted to discussions of the "changing cigarette" (12,255,260). Actually, the recent articles were preceded by earlier ones, e.g., the 1981 SURGEON GENERAL'S report (253), a 1986 HOFFMANN and WYNDER article (254), and a 1990 article by HOFFMANN and HECHT (7).

Several technologies incorporated into commercial products were eventually abandoned because of poor consumer acceptance. One of these was the drastic reduction of tobacco nicotine that resulted in a low, almost zero, MSS nicotine yield. A second was the incorporation of a tobacco substitute that effectively is a diluent for the tobacco. Examples of these include the New Smoking Material® (NSM) from Imperial Tobacco, Cytrel® from Celanese, and the Sutton Smoking Material® (SSM). Each had its own peculiar problem.

Consumers did not accept commercial products containing NSM® or Cytrel® so they were eventually removed from the marketplace. In the NCI Smoking and Health Program on the "less hazardous" cigarette both NSM® and Cytrel® were examined. The biology of NSM® matched the claims made by the manufacturers whereas they did not for Cytrel®. The MSS from Cytrel® cigarettes was found to contain several dozen components not present in tobacco smoke (GREEN *et al*., 261). The data from bioassays conducted on Cytrel® MSS in the NCI program (35) fell far short of those presented by Celanese personnel. While the bioassay results in the NCI program on NSM® MSS were satisfactory, the B*a*P:"tar" ratio was three times that of several popular commercial cigarettes.

## 26 THE US TOBACCO INDUSTRY CRITICIZED: NO NEW CIGARETTE DESIGN TECHNOLOGY SINCE 1975

Recently HOFFMANN and HOFFMANN (12) wrote:

> Major modifications in the makeup of the commercial cigarette were introduced between 1950 and 1975. Since then, there have been no substantive changes toward a further reduction of the toxic and carcinogenic potential of cigarette smoke beyond reducing MS yields of tar, nicotine, and carbon monoxide. Some of these modifications have also resulted in diminished yields of several toxic and carcinogenic smoke constituents.

As mentioned earlier, examination of the graphical representation of the sales-weighed "tar" and nicotine values for US commercial cigarettes reveals that from 1975 to date the FTC "tar" value has decreased from 18 to 11 mg/cig. The HOFFMANNS obviously overlooked the fact that the eight

**Table 16. Effect of the eight significant technologies plus ammoniation on cigarette mainstream smoke properties [a]**

| Smoke component and/or property | Particulate (PP) or vapor (VP) phase | Tobacco blend | Filter tip | | RTS | Paper additive | Filter-tip additive [c] | Air dilution via | | Expanded tobacco | Ammoniation |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | CA [b] | Carbon | | | | Paper porosity | Perforated filter-tip | | |
| FTC "tar" | PP | ⇓ | ⇓ | — | ⇓ | ⇓ | — | ⇓ | ⇓ | ⇓ | — |
| Specific tumorigenicity | PP | ⇓ | — | — | ⇓ | ⇓ | — | ⇓ | ⇓ | ⇓ | — |
| Specific mutagenicity | PP | ⇓ | — | — | — | — | — | — | — | ⇓ | ⇑ |
| Ciliastasis | VP | ⇓ | — | ⇓ | ⇑ | — | — | ⇓ | ⇓ | ⇓ | — |
| FTC nicotine | PP | ⇓ | ⇓ | — | ⇓ | ⇓ | — | ⇓ | ⇓ | ⇓ | ⇓ |
| PAHs | PP | ⇓ | ⇓ | — | ⇓ | ⇓ | — | ⇓ | ⇓ | ⇓ | — |
| Aza-arenes | PP | ⇓ | ⇓ | ⇓ | — | ⇓ | — | ⇓ | ⇓ | ⇓ | — |
| Aromatic amines | PP | ⇓ | ⇓ | ⇓ | — | — | — | ⇓ | ⇓ | ⇓ | — |
| *N*-Heterocyclic amines | PP | ⇓ | ⇓ | ⇓ | — | — | — | ⇓ | ⇓ | ⇓ | — |
| NNAs, volatile | VP | — | — | — | — | — | ⇓ | ⇓ | ⇓ | — | — |
| NNAs, nonvolatile | PP | ⇓ | — | — | — | — | — | ⇓ | ⇓ | — | — |
| TSNAs | PP | ⇓ | ⇓ | ⇓ | — | — | — | ⇓ | ⇓ | ⇓ | — |
| Aldehydes | VP | — | — | ⇓ | ⇑ | — | — | ⇓ | ⇓ | — | ⇓ |
| Phenols | VP | — | — | ⇓ | — | — | ⇓ | ⇓ | ⇓ | — | — |
| Phenols | PP | ⇓ | ⇓ | — | — | — | — | ⇓ | ⇓ | — | — |
| Miscellaneous organic | VP & PP | — | — | — | — | — | — | ⇓ | ⇓ | — | — |
| CO | VP | — | — | — | ⇑ | — | — | ⇓ | ⇓ | ⇓ | — |

[a] ⇓ Indicates property or component may be lowered by appropriate choice of blend, ⇑ indicates property or component may be increased by use of a particular technology.
[a] CA = cellulose acetate.
[c] The filter-tip additive in this case is a plasticizer such as triacetin or Carbowax®.

technologies used in concert and to different degrees have resulted in this change (~40% ) in the FTC "tar" yield from 1975 to date. The decrease in FTC "tar" of more than 50% from 1955 though 1975 attained and surpassed the goal originally proposed by WYNDER in 1957 to resolve the lung cancer situation (36).

Recent claims (see Table 1, Footnote e) that a new cigarette product is the first with lowered levels of carcinogens in its smoke are obviously erroneous. Examination of the sales-weighted average FTC "tar" and nicotine yield from 1955 to date indicates that the "tar" and nicotine have decreased substantially. Correspondingly, the MSS deliveries of B*a*P and other PAHs have decreased not only on a per cigarette basis but also on a per milligram of "tar" delivered basis. This and the decreased specific tumorigenicity of CSC to mouse skin from that observed in 1955 were acknowledged in 1979 by the US SURGEON GENERAL [see pp. 111–112 in (43)].

## 27 TSNAs IN FLUE-CURED TOBACCO: BACK TO THE FUTURE

In a previous section, we discussed the development and utilization of cigarette design technologies that more or less uniformly and simultaneously reduced the levels of toxicants in MSS particulate and vapor phases. After several decades of such activities, examination of the effect of lowering specific tobacco components on the level of specific toxicants in and the biological properties of MSS has once again been implemented. The targets of choice are the TSNAs and the *N*-heterocyclic amines.

As described by WILLIAMS (262) there is general agreement among tobacco scientists that TSNAs are not present in either freshly harvested, i.e., green flue-cured and burley tobaccos. As the tobaccos are cured either by air-curing in the case of burley or in heated barns for flue-cured varieties, the amounts of TSNAs rise to their final levels. In the case of air-curing, the process has changed little for the past fifty years. However, for flue-curing, the process changed drastically in the US during the 1960s and 1970s due to the introduction of energy efficient bulk-curing barns heated directly by the exhaust gases of liquid propane gas or similar burners. It is at this point that a breakdown must have occurred between tobacco agriculturists and chemists. The emission of $NO_2$ during the combustion of liquid propane or natural gas is well known. In fact, the North Carolina Department of Environment and Natural Resources (NCDENR) has electronic spreadsheets available for download from its website that North Carolina industries may use in estimating their $NO_2$ emissions during natural gas or liquid propane combustion. In retrospect, any competent chemist would predict the potential nitrosation of tobacco alkaloids during flue-curing in the presence of combustion exhaust gases. However, without the knowledge of TSNA formation during direct-heating of green tobaccos, the agricultural community adopted the new energy-efficient technique. It appears that prior to this "technological advance", the formation of TSNAs during flue-curing by traditional methods was not a problem.

Rather than using the existing knowledge, at least two research groups during recent years have used the Edisonian approach to discover the problem with direct heating flue-curing of tobacco. PEELE *et al.* (263) demonstrated that modification of the curing process for flue-cured tobacco permitted significant control of its TSNA levels. The curing process was altered from one involving direct-fired burners

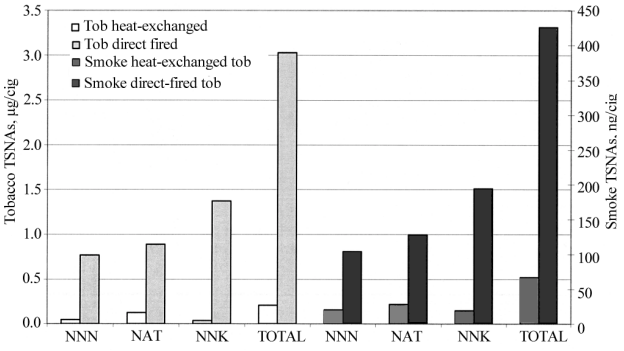



**Figure 3. Comparison of TSNAs in tobacco and smoke of heat-exchanged and direct-fired flue-cured tobaccos**.

to one involving a heat exchange system. During approximately the same time period, WILLIAMS (262) applied for and was granted a US patent on essentially the same modification of the flue-curing barns to achieve the same significant reduction in TSNAs. An example of the TSNA reductions in flue-cured tobacco and its smoke is shown in Figure 3. The tobacco data are taken from WILLIAMS and the smoke data are taken from DOOLITTLE *et al*. (264). As a result of these two disclosures, one through scientific communication and the other through the patent literature, several legal actions have taken place. In May 2001, Star Scientific, Inc., co-founded by Jonnie R. Williams and Francis E. O'Donnell Jr, initiated patent infringement litigation against R.J. Reynolds Tobacco Company, i.e., the employer of PEELE *et al*. Meanwhile, on June 28, 2002, PHILIP MORRIS USA (265) petitioned the US legal system to declare the patent of WILLIAMS *et al*. to be invalid and unenforceable. A recent ruling dismissed the lawsuit of PHILIP MORRIS USA (266).

Regardless of the legal proceedings outcome, two issues arise. On the first issue there appears to be no disagreement from anyone. Discontinuing direct heating for flue curing is desirable from a product stewardship perspective. Every practical effort should be made to reduce the amounts of alleged human carcinogens from tobacco products. However, whether the reduction or elimination of TSNAs from MSS will result in a "less hazardous" cigarette is unknown. Earlier, we have presented pure compound data in Tables 1 and 3 that indicate TSNAs play a minor role in MSS carcinogenesis. Additionally, from a comparison of the biological effect (Neutral Red cytotoxicity, mutagenicity in the Ames test with several *Salmonella typhimurium* strains) of the MS CSCs from flue-cured tobacco cigarettes with normal and reduced levels of TSNAs, DOOLITTLE *et al.* (264) reported no significant difference between the biological activity of the two CSCs. Although the DOOLITTLE *et al.* data appear to support the hypothesis on a whole-smoke basis that MSS TSNAs are of relatively minor toxicological importance, the sensitivity of the Ames assay is not sufficient to differentiate between the cigarettes tested. For example, consider the following points published by DOOLITTLE *et al.*:

- The minimum amount of NNK needed for a mutagenic response in the Ames assay is 200 µg.
- The maximum amount of CSC that can be tested is 250 µg.
- In 250 µg of CSC there is 1.33 and 0.13 ng of NNK from direct fired and heat exchanged flue-cured tobacco, respectively.
- The amount of NNK in the CSC from either flue-cured tobacco smoke is too low for a response.

Just as analytical chemists must keep in mind limits of detection, biologists must also be aware of their assay limits.

A major class of MSS components to attain notoriety recently, the *N*-heterocyclic amines – the so-called Sugimura compounds – were initially identified as components of protein pyrolysates and cooked protein-containing foods. Despite their inordinately high mutagenicity in the Ames test (Table 17), their tumorigenicity to laboratory animals (267), and their inclusion in recent lists of MSS toxicants (255), no *N*-heterocyclic amine in MSS has received the attention of such components as B*a*P or NNK. However, CLAPP *et al*. (268) reported that removal of protein from flue-cured and burley tobacco produces significant reductions in the mutagenicity (Ames test, *Salmonella typhimurium* strains TA98 and TA100) of the CSCs from both reduced protein flue-cured and burley tobacco products.

Pyrolysis of glutamic acid and tryptophan yield several *N*-heterocyclic amines, e.g., Glu-P-1, Glu-P-2, Trp-P-1, and Trp-P-2. These four *N*-heterocyclic amines were subsequently identified not only in cooked foods but also as tobacco smoke components. Their precursors in foods and tobacco smoke are considered to be glutamic acid and tryptophan, either bound in a protein or as the free amino acid.

**Table 17. Mutagenic activities (revertants/µg) of *N*-heterocyclic amines towards *Salmonella typhimurium*** [a]

| Compound (designation) | | TA98 | | TA100 | |
|---|---|---|---|---|---|
| | | Lee *et al*. (165) | Sugimura (166) | Lee *et al*. (165) | Sugimura (166) |
| IQ | {2-amino-3-methylimidazo[4,5-*f*]quinoline} | 222000 | 433000 | 11000 | 7000 |
| MeIQ | {2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline} | 1327000 | 661000 | 70000 | 30000 |
| Glu-P-1 | {2-amino-6-methyldipyrido[1,2-*a*:3',2'-*d*]imidazole} | 73000 | 49000 | 4000 | 3200 |
| Glu-P-2 | {2-aminodipyrido[1,2-*a*:3',2'-*d*]imidazole} | 600 | 1900 | 400 | 1200 |
| Trp-P-1 | {3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole} | 20000 | 39000 | 500 | 1700 |
| Trp-P-2 | {3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole} | — | — | 2000 | 1800 |
| B*a*P | {benzo[*a*]pyrene} | 200 | — | — | — |

[a] Tests with *Salmonella typhimurium* involved use of S-9 mix.

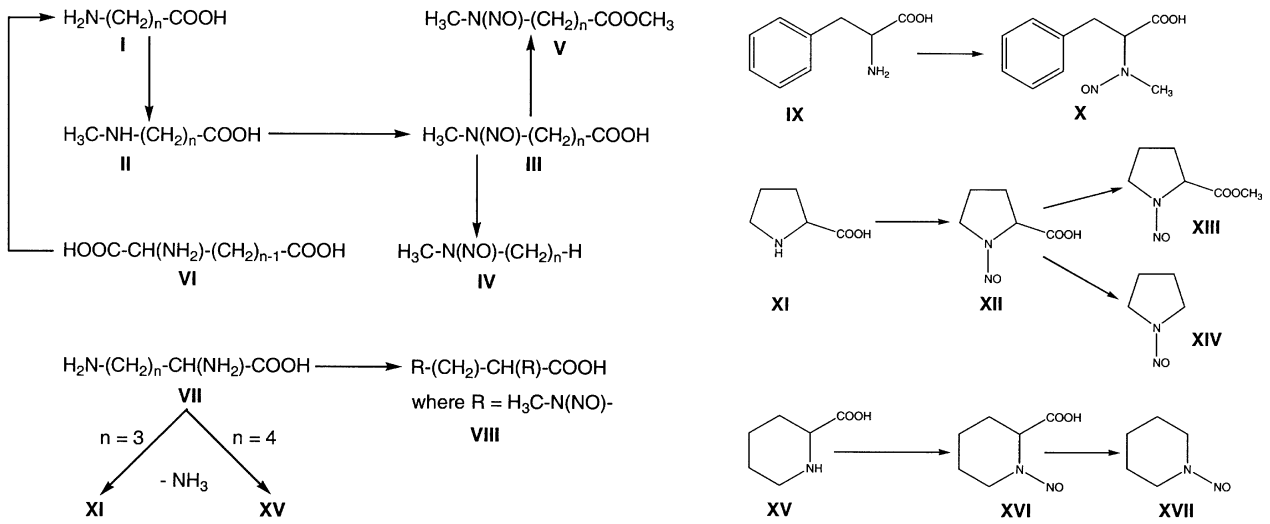



**Figure 4. Relationship among amino acids, *N*-nitrosamino acids, their esters, and *N*-nitrosamines**

*Aliphatic compounds*

| No. | n = 1 | n = 2 | n = 3 | n = 4 |
|---|---|---|---|---|
| I | Glycine [2-aminoacetic acid] | β-Alanine [3-aminopropanoic acid] | 4-Aminobutanoic acid | |
| II | Sarcosine [*N*-methylaminoacetic acid] | 3-(*N*-Methylamino)propanoic acid | 4-(*N*-Methylamino)butanoic acid | |
| III | *N*-Nitrososarcosine [2-(methylnitrosamino)acetic acid] (**NSAR**) | 3-(Methylnitrosamino)-propanoic acid | 4-(Methylnitrosamino)butanoic acid | |
| IV | *N*-Nitrosodimethylamine (**NDMA**) | *N*-Nitrosoethylmethylamine (**NEMA**) | *N*-Nitrosomethylpropylamine (**NMPA**) | |
| V | 2-(Methylnitrosamino)acetic acid, methyl ester | 3-(Methylnitrosamino)-propanoic acid, methyl ester | 4-(Methylnitrosamino)butanoic acid, methyl ester | |
| VI | | | Glutamic acid | |
| VII | | | Ornithine [2,5-diamino-pentanoic acid] | Lysine [2,6-diaminohexanoic acid] |
| VIII | | | 2,5-Di-(methylnitros-amino)pentanoic acid | 2,6-Di-(methylnitros-amino)hexanoic acid |

*Aromatic and heterocyclic compounds*

| | | | | | |
|---|---|---|---|---|---|
| IX | 2-Amino-3-phenylpropanoic acid [phenylalanine] | XII | *N*-Nitrosoproline (**NPRO**) [1-nitroso-2-pyrrolidinecarboxylic acid] | XV | Pipecolic acid [2-piperidinecarboxylic acid] |
| X | 2-(Methylnitrosamino)-3-phenyl-propanoic acid | XIII | 1-Nitroso-2-pyrrolidinecarboxylic acid, methyl ester | XVI | 1-Nitroso-2-piperidine-carboxylic acid |
| XI | Proline [2-pyrrolidinecarboxylic acid] | XIV | *N*-Nitrosopyrrolidine (**NPYR**) | XVII | *N*-Nitrosopiperidine (**NPIP**) |

During smoking, the pyrogenesis of amino acids would be reduced from reduced protein tobacco.

The importance of the role played by amino acids in tobacco (and indirectly the tobacco proteins) as precursors of NNAs in tobacco smoke may be seen by examination of the information in Figure 4. Several amino acids identified in tobacco and/or tobacco smoke are listed in Table 18. At least eight are involved directly or indirectly as precursors of NNAs that account for almost half of the NNAs identified in tobacco and tobacco smoke. Reduction of the levels of these amino acids or the tobacco protein source should reduce the levels of NNAs in tobacco and smoke. Thus, removal or reduction of the levels of the proteins and amino acids in tobacco serves two purposes: a) Reduction of the levels in tobacco smoke of the *N*-heterocyclic amines and b) reduction of the amino acid-derived NNAs in tobacco and smoke.

## 28 DISCUSSION

While we do n ot dispute the inherent risks of cigarette smoking, throughout our review we have tried to put several issues in perspective. The number of MSS toxicants listed by various individuals, institutions, and government agencies has increased steadily over the past few decades. However, with a few exceptions, it is obvious that the exposure of a pack-a-day smoker to the listed MSS toxicants (Tables 1 and 2) is much less than the exposure in the workplace permitted by or acceptable to OSHA (Table 3).

While we have noted the problem inherent in extrapolation of biological effects observed in laboratory animals treated by various administration methods with exaggerated doses of a specific substance to the effect on the smoker inhaling a cigarette MSS aerosol containing a much smaller dose of

**Table 18. Amino acids in tobacco and/or tobacco smoke**

| | |
|---|---|
| α-Alanine | Leucine |
| β-Alanine [a] | Lysine {2,6-diaminohexanoic acid} [a] |
| Aspartic acid | Ornithine {2,5-diaminopentanoic acid} [a] |
| 2-Aminobutanoic acid | Phenylalanine [a] |
| 4-Aminobutanoic acid [a] | Proline [a] |
| Cysteine | Serine |
| Glutamic acid [a,b] | Tryptophan [b] |
| Glycine [a] | Valine |

[a] The amino acid is involved in *N*-nitrosamine formation.
[b] The amino acid is involved in *N*-heterocyclic amine formation.

that substance admixed with nearly 4800 other identified substances (and, as suggested by WAKEHAM (269), possibly as many as 100000 substances), a detailed discussion of the problem is beyond the scope of this paper. Other highly capable authorities have spoken at length to the problem.

The repeated assertion since the advent of the low-"tar" cigarette that increased levels of added ingredients to cigarette tobacco have increased the levels of MSS toxicants and the adverse MSS biological effect is without merit. No evidence to prove such an assertion has ever been presented, but much data contradicting the assertion have been published (16–18,81,82,87). This assertion is reminded of many others made over the years that are not supported by credible evidence (176).

Although we have dealt at length with the many lists of MSS toxicants, we have also questioned why similar lists (Table 11) are seldom generated for MSS components known to counteract or diminish the adverse biological activity of many of the listed MSS toxicants.

Over the years, various individuals, institutions, and agencies opposed to cigarette smoking have not only acknowledged the significance of the Tobacco Industry's development and use of cigarette design technologies to lower the levels in MSS of the toxicants but also commended the Industry for its activities in this regard. However, even investigators as zealous as Wynder and Hoffmann were aware of one of the major problems, if not *the* major problem, in the design of a "less hazardous" cigarette, namely, acceptance by the consumer. When the low-"tar" cigarette had obviously become the choice of many smokers, WYNDER and HOFFMANN, after commending the Tobacco Industry for its emphasis on low-"tar" cigarette marketing, noted (251):

> Development of a less harmful cigarette acceptable to the majority of the smokers needs to continue. We must be realists. A completely safe cigarette smoked by only 1% of the smoking public is of considerably less societal benefit than a cigarette with some adverse effects smoked by 90% of the public.

WYNDER reiterated the above statement on another occasion (270):

> [I]t is important to appreciate that a virtually harmless cigarette smoked by only 1% of the population will have a lesser impact on the reduction of tobacco-related diseases than a somewhat more harmful cigarette smoked by 80% of the total smoking population. Research on the less harmful cigarette should therefore be directed toward developing a cigarette containing the lowest possible amount of harmful elements for all tobacco-related diseases, but one that has sufficient acceptability for the largest segment of smokers.

GORI expressed a similar sentiment in 1977 (271) and in his summary of the 1979 Banbury Conference on the "less hazardous" cigarette (226). However, at that time he also considered that a weaning process – the sequential changing by a smoker to acceptable lower and lower "tar" delivery cigarette brands – would ultimately attain the goal of complete cessation (272).

We have noted that the recent criticism of the Tobacco Industry for its failure to generate any new significant cigarette design technologies since 1975 (12) is totally without merit. The eight design technologies deemed significant (Table 15), when used in concert but to different degrees since 1975, have continued to reduce the sales-weighted FTC "tar" substantially below the goal originally recommended, i.e., a 50% reduction from the mid-1950 "tar" yield (36). None of the critics has ever developed a cigarette design technology to match the significance of the eight in US and worldwide commercial cigarette production since the late 1960s. The attitude of the critics and the contrasting performance by the Industry remind us of a statement by ARISTOTLE (273):

> In practical matters, the end is not mere speculative knowledge of what is to be done, but rather the doing of it.

Four centuries ago, long before the development of our present skills in chemical separations and analyses and toxicological techniques, ROWLAND categorized inhaled and exhaled tobacco smokes as "toxicants" in an epigram (274):

> But this same poyson, steeped India weede,
> In head, hart, lunges, do soote and copwebs breede.
> With that he gasp'd, and breath'd out such a smoke
> That all the standers by were like to choke.

ROWLAND, with absolutely no knowledge of the composition of tobacco smoke, could not define any specific component in it as a "poyson". Despite the tremendous advances made in our chemical and toxicological capabilities (4) plus the lists of MSS toxicants, numerous noted critics of cigarette smoke have expressed reservation about the effect on the smoker of many of the MSS components listed as toxicants. Table 19 provides qualifying statements made not only by HOFFMANN and HECHT on several listed toxicants in the text accompanying their famous "List of 43" (7) but also by others on the biological activity of MSS toxicants.

## 29 CONCLUSIONS

- In terms of developing a "less hazardous" cigarette (LHC), one needs to define the reference point. If we compare commercial brands of today's cigarettes with those in the marketplace during the 1950s, then there is no question that LHCs have already been produced. However, the more important question is whether or not we can in the future develop LHCs than those in the marketplace today. This is the challenge facing the Tobacco Industry. We are optimistic that this goal can be achieved.
- Paramount among the criteria for new products is consumer acceptability. Regardless of the means to produce

**Table 19. Comments by various authorities on listed MSS toxicants**

| MSS component | CAS no. | Comment | References |
|---|---|---|---|
| Benzo[*a*]pyrene | 50-32-8 | [T]he tarry condensates of the smoke obtained by smoking cigarettes in machines . . . have 3,4-benzpyrene but the amount is exceedingly small and there is considerable doubt about whether the concentration is high enough to produce carcinogenic action. | Cook [a] (275) |
| | | 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. | Coultson (276) |
| | | But 30 years of laboratory research has yet to identify reliably the important carcinogenic factors in cigarette smoke. | Peto and Doll [b] (277) |
| | | This complexity [of tobacco smoke] has made it difficult to identify any individual agent within tobacco smoke as the chief cause of any of the diseases that are caused by smoking . . . | IARC (44) |
| NNK | 64091-91-4 | It [NNK] has not been tested by inhalation. | Hoffmann and Hecht (7) |
| | | Relevant information not available [on this compound]. | OSHA (278) |
| Aniline, 2-methyl- | 95-53-4 | Recent studies have . . . shown that single ring aromatic amines, including the weak bladder carcinogen *o*-toluidine [2-toluidine, 2-methylaniline] are present in human urine . . . The available data do not indicate that there are significant differences between smokers and nonsmokers. | Hoffmann and Hecht (7) |
| Naphthalene, 2-amino- | 91-59-8 | 2-[N]aphthylamine [has] been reported in tobacco or tobacco smoke. [That] compound is a bladder carcinogen in man . . . , but is present in cigarette smoke in amounts (22 ng/cigarette) too low to be considered a health hazard. | Schmeltz and Hoffmann (279) |
| | | The presence of β-naphthylamine [2-aminonaphthalene] in cigarette smoke has been demonstrated . . . , along with other carcinogenic aromatic amines . . . The yield is so low that the [the researchers] did not believe these agents contributed to the risk of bladder cancer in smokers. | USPHS [see p. 41 in (253)] |
| | | On the basis of quantitative data for aromatic amines in cigarette smoke, an etiological significance of these traces of carcinogenic amines in bladder cancer is questionable . . . | USPHS [see pp. 207–208 in (172)] |
| Benzene | 71-43-2 | Concern has been expressed in recent years about the possible risk of leukemia for workers who have been exposed to benzene . . . Although some prospective and retrospective studies have reported a somewhat higher risk of leukemia for cigarette smokers, these data remain unconfirmed and no dose-response relationship has been established between death rate for leukemia and number of cigarettes smoked. | USPHS [see p. 51 in (43)] |
| Acrylonitrile | 75-05-8 | Although it is present in cigarette [MSS], its role in tobacco carcinogenesis is difficult to evaluate due to lack of data. | Hoffmann and Hecht (7) |
| Vinyl chloride | 75-01-4 | Its low levels in cigarette [MSS] do not support a major role in tobacco carcinogenesis | Hoffmann and Hecht (7) |
| Cadmium | 7440-43-9 | The possible roles of chromium, cadmium, and lead in tobacco carcinogenesis are difficult to evaluate given the present data base . . . Taken together, the evidence for a major role of these materials as etiologic factors in tobacco carcinogenesis is not compelling. | Hoffmann and Hecht (7) |
| Chromium | 7440-47-4 | | |
| Lead | 7439-92-1 | | |
| Nickel | 7440-02-0 | It is not likely that nickel plays a significant role in the etiology of lung cancer in cigarette smokers. | USPHS [see p. 200 in (172)] |
| Polonium-210 | 7440-08-6 | The quantities of polonium-210 found in the lungs of smokers are generally about three times higher than those in nonsmokers. However, the significance of polonium-210 in tobacco-induced lung cancer has been questioned upon comparison of these data with those obtained in miners. | Hoffmann and Hecht (7); Harley *et al*. (77) |
| | | In the case of polonium-210, a recent indepth [*sic*] study raises doubts on the significance of $^{210}$Po as a factor contributing to lung cancer in smokers. | USPHS [see p. 94 in (43)] |
| | | [P]olonium-210 is present in tobacco and tobacco smoke (0.03 to 1.0 pCi/cigarette); however, it is unlikely that these traces represent a major risk for the smoker. | USPHS [see p. 211 in (172)] |

[a] Cook and his colleagues isolated benzo[*a*]pyrene from coal tar, identified it, and demonstrated its carcinogenicity to mouse skin (280).

[b] Doll was the author of one of the 1950 retrospective studies on smoking and lung cancer (2).

LHCs, if people will not smoke them, then the effort is useless.

- For most of the last century, the chemistry of tobacco and its smoke has been at the forefront of developing improved smoking products; however, this appears to be changing. The analogy of the forest and trees seems to fit this situation. With chemistry, we can only look at individual MSS or groups of MSS constituents, i.e., the trees, at a time while biological assays within their limitations survey a broad range of effects, i.e., the forest. Ultimately we are not concerned with the health effects related to exposure to individual chemicals in smoke but rather to their effects as a mixture. It is beyond the scope of today's knowledge to predict the toxicology of a complex mixture from data on its individual components.
- Results of MSS chemical studies may be used to identify toxicants of concern and they can also quantify the amounts these constituents. However, neither the toxicology of the individual chemicals nor whether they have been reduced can be the basis for a "less hazardous" claim. Many scientists will claim that if we removed all the PAHs and TSNAs from MSS then a LHC would result. This is a true statement on an absolute and product stewardship basis, but whether it will make a meaningful improvement is unknown.
- Although approximately 4800 components of MSS are known, there are toxicological data on only a few hundred of these chemicals. Because of this great unknown, the Tobacco Industry's course of reducing MSS "tar" has been a prudent action.
- Scientists have hypothesized and there has been much public hoopla over the potential detrimental effects of tobacco additives and their pyrolysis products. However, we are not aware of a single scientific study that confirms the alleged adverse effects. To the contrary, there are several excellent, comprehensive studies that fail to demonstrate toxicological problems with tobacco additives.
- Reduction of vapor-phase constituents, e.g., carbon monoxide, has also been successful, but it appears that the great challenge especially to western countries is to develop consumer acceptable charcoal-filtered cigarettes or their equivalent. Many of the MSS toxicants ranking high in our quantitative risk assessments, e.g., acrolein, VNNAs, can be effectively removed by selective filtration. Some prominent scientists have hypothesized that a major factor influencing the differential lung cancer rate between Japanese and western smokers is the great popularity of charcoal-filtered cigarettes in Japan. This appears to be a situation where the MSS chemistry, biological assays, and epidemiological studies are in agreement, i.e., reducing MSS vapor-phase toxicants is beneficial.
- The modification of flue-curing barns from direct heating to heat exchanging appears to be a simple method to reduce TSNAs in flue-cured tobacco. Two questions arise from this change: 1) Whose idea was it to use direct-fired flue-curing barns and do they have any liability for creating a "more hazardous" cigarette, and 2) Does reducing the TSNAs make any biologically significant difference? The existing data do not support the contention that reducing TSNAs makes a meaningful difference. Of course, if this last statement be correct, then the answer to the first question is moot.
- Among the most intriguing research that we have encountered during this review is the seemingly beneficial effects of removing protein from tobacco. Perhaps the commercialization of deproteinized smoking tobacco is beyond the realm of feasibility, but with all the tools of modern agricultural science to produce custom-made crops, it would appear to be a fertile field of research.

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## 31 GLOSSARY

Abbreviations

| | | |
|---|---|---|
| 1R4F | = | Kentucky Reference Cigarette |
| A$\alpha$C | = | 2-amino-9*H*-pyrido[2,3-*b*]indole |
| ADC | = | average daily concentration |
| ACGIH | = | American Conference of Governmental Industrial Hygienists, Inc. |
| ADI | = | acceptable daily intake |
| AEF | = | aqueous ethanol-soluble fraction |
| AM | = | antimutagenicity test |
| $As_2O_3$ | = | arsenious oxide; arsenic trioxide |
| AT | = | antitumorigenicity test |
| B*a*A | = | benz[*a*]anthracene |
| B*a*P | = | benzo[*a*]pyrene |
| B*e*P | = | benzo[*e*]pyrene |
| CA | = | cellulose acetate |
| CAS | = | Chemical Abstract Service |
| CCHE | = | Center for Children's Health and Environment |
| CDD | = | chlorodibenzo-*p*-dioxin |
| CDF | = | chlorodibenzofuran |
| CO | = | carbon monoxide |
| CPDB | = | Carcinogenic Potency Database |
| CPSC | = | Consumer Product Safety Commission |
| CSC | = | cigarette smoke condensate |
| CORESTA | = | Centre de Coopération pour les Recherches Scientifiques relatives au Tabac |
| DBA | = | dibenz[*a*,*h*]anthracene |
| DB*a*,*i*P | = | dibenzo[*a*,*i*]pyrene = benzo[*rst*]pentaphene |
| DDE, *p*,*p*'- | = | 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethene |
| DDT, *p*,*p*'- | = | 1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethane |
| DHHS | = | Department of Health and Human Services |
| DMBA | = | 7,12-dimethylbenz[*a*]anthracene |
| DMH | = | dimethylhydrazine |

DNA = deoxyribonucleic acid
EC = ethyl carbamate
EPA = Environmental Protection Agency
EPCRA = Emergency Planning and Community Right-to-Know Act
ERGO = ERGO Forschungsgesellschaft mbH, Hamburg
ETS = environmental tobacco smoke
FTC = Federal Trade Commission
GRAS = generally recognized as safe
Glu-P-1 = 2-amino-6-methyldipyrido[1,2-*a*:3',2'-*d*]imidazole
Glu-P-2 = 2-aminodipyrido[1,2-*a*:3',2'-*d*]imidazole
HCl = hydrochloride
HCN = hydrogen cyanide
HEAST = Health Effects Summary Table
HERP = Human Exposure to Rodent Potential
HI = hazard index
HTML = hypertext markup language
IARC = International Agency for Research on Cancer
ILCR = incremental lifetime cancer risk
INBIFO = INBIFO Institut für Biologische Forschung, Köln, Germany
i.p. = intraperitoneal injection
IQ = 2-amino-3-methyl-3*H*-imidazo[4,5-*f*]quinoline
IRIS = Integrated Risk Information System
ISO = International Standards Organization
IUPAC = International Union of Pure and Applied Chemistry
IURF = Inhalation Unit Risk Factor
LHC = "less hazardous" cigarette
M = mouse
7-MBA = 7-methylbenz[*a*]anthracene
12-MBA = 12-methylbenz[*a*]anthracene
MC = 3-methylcholanthrene
= 1,2-dihydro-3-methylbenz[*j*]aceanthrylene
MeA$\alpha$C = 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole
5-MeC = 5-methylchrysene
MeIQ = 2-amino-3,4-dimethyl-3*H*-imidazo[4,5-*f*]quinoline
MeIQx = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline
MS = mainstream
MSS = mainstream smoke
MTD = maximum tolerated dose
NAAC = *N*-nitrosamino acid
NAB = *N*'-nitrosoanabasine
NAT = *N*'-nitrosoanatabine
NATA = National-scale air toxics assessment
NCDENR = North Carolina Department of Environment and Natural Resources
NCI = National Cancer Institute
NDEA = *N*-nitrosodiethylamine
NDELA = *N*-nitrosodiethanolamine
NDMA = *N*-nitrosodimethylamine
NEMA = *N*-nitrosoethylmethylamine
NIOSH = National Institute of Occupational Safety and Health
NNA = *N*-nitrosamine
iso-NNAC = 4-(*N*-methylnitrosamino)-4-(3-pyridinyl)butyric acid
NNAL = 4-(*N*-methylnitrosamino)-1-(3-pyridinyl)-1-butanol
NNK = 4-(*N*-methylnitrosamino)-1-(3-pyridinyl)-1-butanone
NNN = *N*'-nitrosonornicotine
NO = nitric oxide
NPRO = *N*-nitrosoproline
NPYR = *N*-nitrosopyrrolidine
NSM = New Smoking Material
ORNL = Oak Ridge National Laboratory
OSHA = Occupational Safety and Health Administration
PAH = polycyclic aromatic hydrocarbon
PCB = polychlorinated biphenyl
PCDD = polychlorodibenzo-*p*-dioxin
PCDF = polychlorodibenzofuran
pCi = picocurie
PEL = permissible exposure level
PhIP = 2-amino-1-methyl-6-phenyl-1*H*-imidazo[4,5-*b*]pyridine
PHS = Public Health Service
PM = Philip Morris or particulate matter
p.o. = per os (by mouth)
$^{210}$Po = polonium-210
PP = particulate phase
R = rat
RAIS = Risk Assessment Information System
RfC = reference concentration
RJR, RJRT = R.J. Reynolds Tobacco Co.
RTECS = Registry of Toxic Effects of Chemical Substances
RTS = reconstituted tobacco sheet
S-9 = a rat liver homogenate fraction used to enhance mutagenesis detection
s.c. = subcutaneous injection
SSM = Sutton Smoking Material
SSS = sidestream smoke
STEL = short-term exposure limit
TA98 = *Salmonella typhimurium* strain
TA1538 = *Salmonella typhimurium* strain
TBA = tumor-bearing animal
TCCD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin
$TD_{50}$ = tumor development in 50% of animals tested
TDE, *p,p'*- = 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethane
TEQ = toxicity equivalent
TLV = Threshold Limit Value
TPM = total particulate matter
Trp-P-1 = 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole
Trp-P-2 = 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole
TSNA = tobacco-specific *N*-nitrosamine
$TWA_8$ = 8-hour time weighted average
TWG = Tobacco Working Group
UK = United Kingdom
URF = Unit Risk Factor
US = United States
USA = United States of America
USDA = United States Department of Agriculture
USPHS = United States Public Health Service
VNNA = volatile *N*-nitrosamine
VP = vapor phase
WHO = World Health Organization
WTPM = wet total particulate matter

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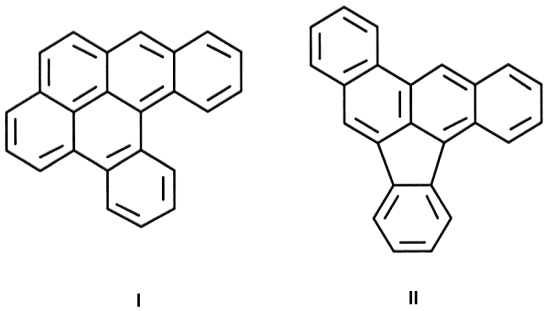
APPENDIX. Tobacco smoke components not listed or seldom listed as toxicants

In the body of our report we have discussed in considerable detail the many lists of MSS toxicants, most of which were issued over the past few decades. Obviously, various investigators, institutions and government agencies have been extremely zealous in their generation of such lists. We also pointed out the fact that most of the compilers of the lists persist in including MSS components that are no longer relevant since their precursors have not been present in the cigarette filler for decades, components whose presence in MSS is highly suspect, and components for which no or extremely few quantitative analytical data exist. In addition, the compilers persist in listing ranges for cigarette yields of many toxicants and the ranges include analytical data, whether acceptable or poor, generated on cigarettes manufactured in the 1950s and 1960s. Such data are totally irrelevant to cigarettes manufactured during the past two decades.

Between the mid-1950s and late 1970s extensive research was conducted both within the Tobacco Industry and outside it to define the components in tobacco and its smoke and the relationship between them. Much of the early work revolved around the presence of PAHs, particularly B*a*P, in cigarette MSS. Eventually, interest in the PAHs (and B*a*P) declined and was replaced with interest in NNAs. This interest became even more intense with the discovery in tobacco and smoke of TSNAs, especially NNK. Except for efforts to control the levels of TSNAs in tobacco and smoke, much of the research to define the composition of tobacco smoke declined within the Tobacco Industry and outside it. In our main report, we pointed out some of the problems with the current emphasis on TSNAs and NNK.

While the number of MSS components listed as toxicants has grown steadily, particularly since the IARC 1986 report on smoking (1), numerous other MSS components classified in the literature as toxicants have been omitted from the MSS toxicant lists with no specific reason offered for their omission. In the main part of this report we have written at some length on the omission of the dioxins from all but one list of cigarette MSS toxicants. Even though the precise MSS levels of other omitted toxicants have not been quantified probably because they are extremely low, most of them have been reported as tumorigenic to laboratory animals. Lack of knowledge of per cigarette MSS levels is no excuse for omission. In the lists of tobacco smoke components first categorized as "IARC Group 2A carcinogens" or "IARC Group 2B carcinogens", the levels of several were originally recorded in the "IARC Group 2B" category only as "present" or "present in trace amounts" (2). These included benzo[*b*]furan, caffeic acid, dibenzo[*a,e*]pyrene {naphtho[1,2,3,4-*def*]chrysene}, and the much discussed PAH dibenzo[*a,l*]pyrene {dibenzo[*def,p*]pyrene}.

The constituent first reported in the late 1950s as the $C_{24}H_{14}$ PAH 1,2,3,4-dibenzopyrene, later named dibenzo[*a,l*]pyrene and then dibenzo[*def,p*]pyrene (**I**), (3–7) was subsequently shown in 1966 to be the isomeric dibenz[*a,e*]aceanthrylene (**II**) (also known as dibenzo[*a,e*]fluoranthene) (8) (see Appendix Figure 1). The authentic dibenzo[*a,l*]pyrene was subsequently identified in MSS (9).



I II

**Appendix Figure 1. Dibenzo[*a,l*]pyrene {dibenzo[*def,p*]chrysene (I), dibenz[*a,e*]aceanthrylene {dibenzo[*a,e*]fluoranthene} (II)**

We find the dibenzo[*a,l*]pyrene-dibenzo[*a,e*]aceanthrylene situation to be an interesting one. Several investigators reported dibenzo[*a,l*]pyrene to be present in MSS, others reported MSS yields from various late 1950s cigarettes, e.g., 16 ng/cig by LYONS (4), 0.02 ng/cig by VAN DUUREN (5), 0.6 ng/cig by RODGMAN and COOK (6). When the identity error was resolved by LAVIT-LAMY and BUU-HOÏ (8) in 1966 and acknowledged by HOFFMAN and WYNDER (10) and others involved in tobacco smoke composition studies, some agencies, particularly IARC (1), persisted in listing dibenzo[*a,l*]pyrene as present in MSS, using the 1958 VAN DUUREN report (5) as authority. The yield data, such as they are from the late 1950s, were for dibenz[*a,e*]aceanthrylene not for dibenzo[*a,l*]pyrene. The per cigarette yield ranged from 0.02 to 16 ng/cig. Despite the range and the knowledge that the MSS component dibenz[*a,e*]aceanthrylene (**II**) is tumorigenic to mouse skin and a known initiator (11), it is never listed as an MSS toxicant. Dibenzo[*a,l*]pyrene with similar biological properties, no quantitative yield data, and only described as present is repeatedly listed as a significant tumorigen in MSS. As we noted in our main report, the paucity of data on dibenzo[*a,l*]pyrene vs. the wealth of data on B*a*P raises serious questions as to why they both are considered equally significant tumorigens in cigarette MSS.

While benzo[*a,l*]pyrene has been included in toxicant list after list, HECHT (12) commented that its presence in cigarette smoke has not been confirmed. We feel that one has to weigh HECHT's comment against the current status of defined MSS composition. Since the appreciable decline in detailed MSS composition studies after the late 1970s, no one or no group has attempted to confirm the identities of the great number of PAHs (9,13,14), aza-arenes (15,16), nitrogen-containing components (19), or ether- (18) and water-soluble components (19) newly reported in cigarette MSS in the 1970s. Examination of the post-1980 literature indicates that the identities of nearly half the components described in those studies have never been confirmed. Because of that, would HECHT also discount their presence in MSS in the same way he discounts the presence of benzo[*a,l*]pyrene?

In some ways the inclusion or exclusion of a specific toxicant from a list appears to be somewhat of a "copy cat" syndrome. For example, the 1986 HOFFMANN-HECHT list (20) includes chrysene but not *N*-nitrosodi-*n*-propylamine or *N*-nitrosodi-*n*-butylamine. The 1994 OSHA (21) list omits chrysene but includes the two NNAs. In their 1997 list (22), HOFFMANN and HOFFMANN include the two NNAs but not chrysene.

In Appendix Table 1 we have listed over two dozen other MSS components demonstrated to exhibit adverse biological properties in one or more species of laboratory animals that are seldom, if ever, included in lists of MSS toxicants.

From the mid-1950s to the late 1970s, the number of completely or partially identified PAHs in cigarette MSS increased from very few in 1954 (54) to over 90 by 1964 (55) to more than 500 reported from the seminal study by SNOOK *et al.*, USDA personnel at Athens, GA (9,13,14). A somewhat similar chronological situation existed with the number of identified aza-arenes in MSS from the mid-1950s to 1981 when the list of identified aza-arenes was expanded by HECKMAN and BEST (17) and again by the USDA, Athens, GA personnel (15). However, the situation is completely different with the NNAs.

During extensive investigations of the composition of tobacco smoke in general and cigarette MSS in particular, much effort was expended in the early 1960s to define the nature of *N*-nitrosation during curing and the smoking process. As more and more NNAs were identified in tobacco and/or tobacco smoke, they were categorized as follows: Volatile NNAs, nonvolatile NNAs,

**Appendix Table 1. Cigarette mainstream smoke components with reported biological activity, including some with tumorigenic properties**

| Tobacco smoke component | CAS no. | References | Biological activity |
|---|---|---|---|
| | | *Polycyclic aromatic hydrocarbons* | |
| Anthracene, 9,10-dimethyl- | 781-43-1 | Rothwell and Whitehead (23) | Dipple *et al*. (24) [a] |
| Benz[*a*]anthracene, 7,12-dimethyl- | 57-97-8 | Pietzsch (25) | Hartwell (26) [b]; Shubik and Hartwell (27) [b] |
| Benz[*a*]anthracene, ethyl- | 31632-62-9 | Lee *et al*. (28) | Dipple *et al*. (24) {at least two of the ethylbenz[*a*]anthracenes are tumorigenic to mouse skin} |
| Benz[*a*]anthracene, 5-methyl- | 2319-96-2 | Bonnet and Neukomm (29) | Dipple *et al*. (24) |
| Benz[*a*]anthracene, 6-methyl- | 316-14-3 | Lee *et al*. (28) | Dipple *et al*. (24) |
| Benz[*a*]anthracene, 8-methyl- | 2381-31-9 | Lee *et al*. (28) | Dipple *et al*. (24) |
| Benz[*a*]anthracene, trimethyl- | 60826-78-0 | Lee *et al*. (28); Snook *et al*. (13,14) | Dipple *et al*. (24) {several trimethylbenz[*a*]anthracenes are tumorigenic to mouse skin} |
| Benzo[*c*]phenanthrene | 195-19-7 | Van Duuren (5); Snook *et al*. (9) | Dipple *et al*. (24) |
| Benzo[*c*]phenanthrene, methyl- | | Brunnemann and Hoffmann (30); Van Duuren (5) | Dipple *et al*. (24) {several methylbenzo[*c*]phenanthrenes are tumorigenic to mouse skin} |
| Benzo[*b*]triphenylene | 215-58-7 | Snook *et al*. (9) | Dipple *et al*. (24) |
| Dibenz[*a,e*]aceanthrylene | 5385-75-1 | Wynder and Wright (3); Lyons (4); Van Duuren (5); Rodgman and Cook (6); Pyriki (7) | IARC (11) |
| Dibenz[*a,j*]anthracene | 224-41-9 | Snook *et al.* (9) | Dipple *et al*. (24) |
| 13*H*-Dibenzo[*a,i*]fluorene | 239-60-1 | Lyons and Johnston (31); Pyriki (7) | Hartwell (26) |
| Phenanthrene, 1,2,3,4-tetramethyl- | 71607-70-0 | Snook *et al*. (13) | Hartwell (26) |
| | | *Aza-arenes* | |
| Benz[*a*]acridine | 225-11-6 | Rothwell and Whitehead (23,32); Grimmer *et al*. (33) | IARC (1) |
| Benz[*c*]acridine | 225-51-4 | Rothwell and Whitehead (23,32); Snook *et al*. (15) | IARC (1) |
| Benz[*c*]acridine, 7,9-dimethyl- | 963-89-3 | Klimisch and Beiss (34) | Dipple *et al*. (24) |
| Benz[*c*]acridine, 7,10-dimethyl- | 2381-40-0 | Klimisch and Beiss (34) | Dipple *et al*. (24) |
| Benz[*c*]acridine, 7-methyl- | 3340-94-1 | Grimmer *et al*. (33) | Dipple *et al*. (24) |
| | | *Amines* | |
| Aniline, 2-methoxy- | 90-04-0 | Pailer *et al*. (35) | IARC (1) |
| | | *Phenols* | |
| Catechol, 3-methyl- | 488-17-5 | Brunnemann *et al*. (36) | IARC (37) [c] |
| Catechol, 4-methyl- | 452-86-5 | Brunnemann *et al*. (36) | IARC (1) |
| Eugenol | 97-53-0 | Rodgman and Cook (38) | NTIS (39) |
| Isoeugenol | 97-54-1 | Rodgman and Cook (38) | NTIS (40) |
| | | *Quinones* | |
| 1,4-Benzoquinone | 106-51-4 | Bonnet and Neukomm (29); Schmeltz *et al*. (41) | Takizawa (42) Tiedemann (43) |
| 1,2-Naphthoquinone | 542-42-5 | Benner *et al*. (44) | Takizawa (42) |
| 1,4-Naphthoquinone | 130-15-4 | Schmeltz *et al*. (41); Snook *et al*. (45) | Takizawa (42) |
| | | *Carbohydrates* | |
| Fructose | 57-48-7 | Kobashi and Sakaguchi (46) | Takizawa (47) |
| Glucose | 26655-34-5 | Kobashi and Sakaguchi (46) | Takizawa (47,48) |
| | | *Miscellaneous compounds* | |
| Chloroform | 67-66-3 | Holzer *et al*. (49) | IARC (50) |
| Coumarin | 91-64-5 | Grob and Völlmin (51) | IARC (37,52) |
| Maleic anhydride {2,5-furandione} | 108-31-6 | Schumacher *et al*. (19) | IARC (37) |
| Maleic anhydride, 2,3-dimethyl- | 766-39-2 | Schumacher *et al*. (19) | IARC 37) |
| Succinic anhydride | 108-30-5 | Schumacher *et al*. (19) | IARC (37,53) |

[a] Dipple *et al*. (24) have a tabulation of the tumorigenicity to mouse skin of a wide variety of PAHs and aza-arenes.
[b] Hartwell (26) and Shubik and Hartwell (27) have numerous references to the tumorigenicity of this PAH.
[c] IARC (37) lists 3-methylcatechol, 4-methylcatechol, maleic anhydride, 2,3-dimethylmaleic anhydride, succinic anhydride, and coumarin as biologically activity components of cigarette MSS.

TSNAs, and *N*-nitrosamino acids. Within these four categories, only about 40 NNAs have been identified to date as tobacco and/or tobacco smoke components. Except for an excursion into the identification of *N*-nitrosamino acids, identification of NNAs in MSS almost ceased when NNK and to some extent NNN became the toxicants of choice. This situation raises the question: If a detailed study similar to those conducted on the PAHs and aza-arenes were conducted, how many additional NNAs could be identified in tobacco and/or tobacco smoke?

Appendix Table 2 lists several NNAs reported as tobacco components that are seldom discussed. To date, none of them has been identified in tobacco smoke.

Appendix Table 3 lists the NNAs from which those usually classified as toxicants are selected. Of the NNAs in MSS defined as volatile NNAs, 11 are *N*-nitrosodialkylamines.

In Appendix Table 4 are listed 22 dialkylamines, identified in tobacco and/or smoke as the amine or the NNA. While Appendix Table 4 is not necessarily complete, it suffices for the following discussion: For four NNAs (*N*-nitrosoisobutylmethylamine, *N*-nitrosoethylpropylamine, *N*-nitrosoethylisobutylamine, *N*-nitroso-*n*-butylethylamine), the corresponding amines have not been identified in tobacco smoke. It is highly probable that the four amines are present as MSS components. Alternatively, NNAs corresponding to the other ten dialkylamines identified in tobacco

**Appendix Table 2. *N*-Nitrosamines in tobacco and/or tobacco smoke**

| *N*-Nitrosamine | CAS no. | Identified in tobacco (T) and/or smoke (S) | | Activity |
|---|---|---|---|---|
| | | T | S | |
| 1-Nitroso-2-azetidinecarboxylic acid | 55556-98-4 | x | — | |
| 4-(*N*-Methylnitrosamino)-1-(3-pyridinyl)butanone oxide | 76014-82-9 | x | — | (+) [a] |
| 1-Nitroso-4-hydroxyproline | 2443-30-3 | x | — | |
| 1-Nitroso-3-piperidinecarboxylic acid | 65445-62-7 | x | — | |
| 1-Nitroso-4-piperidinecarboxylic acid | 6238-69-3 | x | — | (–) [173] [b] |
| 3-Nitroso-4-thiazolidinecarboxylic acid | 88381-44-6 | x | — | |

[a] Bioassay results reported by Castonguay *et al.* (56).
[b] Bioassay results in laboratory animals are summarized in Preussmann and Stewart (57). (+) indicates tumor induction, (–) indicates negative response. Number in [ ] represents catalog number in Preussmann and Stewart (57).

**Appendix Table 3. *N*-Nitrosamines in tobacco and/or tobacco smoke**

| Name commonly used | | CAS no. | Activity |
|---|---|---|---|
| | *Tobacco-specific* N-*nitrosamines* | | |
| **4-(*N*-Methylnitrosamino)-1-(3-pyridinyl)butanone** [a] | {NNK} | 64091-91-4 | (+) [98] [b] |
| ***N'*-Nitrosoanabasine** | {NAB} | 37620-20-5 | (+) [185] |
| ***N'*-Nitrosoanatabine** | {NAT} | 71267-22-6 | |
| ***N'*-Nitrosonornicotine** | {NNN} | 16543-55-8 | (+) [154] |
| 4-(*N*-Methylnitrosamino)-4-(3-pyridinyl)butanal | {NNA} | 14091-90-3 | (+) [100] |
| 4-(*N*-Methylnitrosamino)-4-(3-pyridinyl)butanoic acid | {*iso*-NNAC} | 123743-84-0 | |
| 4-(*N*-Methylnitrosamino)-1-(3-pyridinyl)butanol | {NNAL} | 59578-66-4 | (+) [c] |
| 4-(*N*-Methylnitrosamino)-4-(3-pyridinyl)butanol | {*iso*-NNAL} | | |
| | *Volatile* N-*Nitrosamines* | | |
| ***N*-Nitrosodiethylamine** | {NDEA} | 55-18-5 | (+) [7] |
| ***N*-Nitrosodimethylamine** | {NDMA} | 62-75-9 | (+) [1] |
| ***N*-Nitrosodi-*n*-butylamine** | {NDBA} | 924-16-3 | (+) [36] |
| ***N*-Nitrosodi-*n*-propylamine** | {NDPA} | 621-64-7 | (+) [21] |
| ***N*-Nitrosoethylmethylamine** | {NEMA} | 10595-95-6 | (+) [52] |
| ***N*-Nitroso-*n*-butylmethylamine** | {NMBA} | 7068-83-9 | (+) [71] |
| ***N*-Nitrosopiperidine** | {NPIP} | 100-75-4 | (+) [160] |
| ***N*-Nitrosopyrrolidine** | {NPYR} | 930-55-2 | (+) [146] |
| *N*-Nitrosomethylpropylamine | {NMPA} | 924-46-9 | (+) [66] |
| *N*-Nitrosoisopropylmethylamine | | | |
| *N*-Nitrosoethylpropylamine | | 25413-61-0 | |
| *N*-Nitrosoisobutylmethylamine | | 2504-18-9 | |
| *N*-Nitrosoethylisobutylamine | | 71607-99-3 | |
| *N*-Nitrosomorpholine | {NMOR} | 59-89-2 | (+) [192] |
| 3-(Methylnitrosamino)propionaldehyde | | | |
| 3-(Methylnitrosamino)propionitrile | | | |
| | *Nonvolatile* N-*Nitrosamines* | | |
| ***N*-Nitrosodiethanolamine** | {NDELA} | 1116-54-7 | (+) [11] |
| *N*-Nitrosoproline | {NPRO} | 7519-36-0 | (+) [151] |
| | N-*Nitrosamino Acids, Esters, Nitriles* | | |
| *N*-Nitrosarcosine {*N*-methyl-*N*-nitrosoglycine} | {NSAR} | 13256-22-9 | (+) [64] |
| 2-(Methylnitrosamino)acetic acid, methyl ester | | | |
| 4-(Methylnitrosamino)butanoic acid | | 61445-55-4 | |
| 4-(Methylnitrosamino)butanoic acid, methyl ester | | | |
| 2,5-Di-(methylnitrosamino)pentanoic acid | | | |
| 2,6-Di-(methylnitrosamino)hexanoic acid | | | |
| 1-Nitroso-2-piperidinecarboxylic acid | | 4515-18-8 | (–) [172] |
| *N*-Methyl-*N*-nitroso-β-alanine | | 10478-42-9 | |
| 3-(Methylnitrosamino)propanoic acid, methyl ester | | | |
| 2-(Methylnitrosamino)-3-phenylpropanoic acid | | | |
| *N*-Nitrosoproline, methyl ester | | | |

[a] Compounds listed in bold are included in Table 1 of our main report.
[b] (+) indicates tumor induction; (–) indicates no response. Number in [ ] represents catalog number in Preussmann and Stewart (57).
[c] See Castonguay *et al*. (56).

and/or tobacco smoke have not yet been identified in smoke, e.g., no NNA corresponding to *sec*-butylmethylamine, isopentylmethylamine, or isopropylidenemethylamine identified as MSS components has been identified in MSS. Synthetically, the corresponding NNAs are as easily prepared as *N*-nitrosodimethylamine or *N*-nitrosodiethylamine so their pyrogenesis during the smoking process should not be hindered. Thus, it is highly probable that the ten NNAs are present in tobacco smoke. For each of the six NNAs listed as toxicants in Table 1 of the main body of our report, the corresponding amine has been identified in tobacco and/or tobacco smoke.

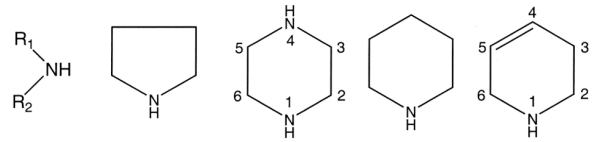
Many other secondary amines have been identified in tobacco smoke but for most of them no corresponding NNA has been identified in smoke. These include a series of *N*-substituted anilines, all amenable to *N*-nitrosation. Others include the alkyl derivatives of pyrrolidine (**IV**), piperazine (**V**), and piperidine (**VI**) (Appendix Figure 2). The amines pyrrolidine (**IV**), piperidine (**VI**), and 1,2,3,6-tetrahydropyridine (**VII**) have been identified in cigarette MSS but not piperazine (**V**).

For each of the piperazines, mono- and di-*N*-nitroso derivatives are possible. In many instances, the NNAs are readily synthesized and have been tested for tumorigenicity [see PREUSSMANN and STEWARD (57)].

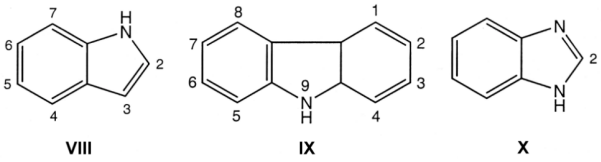
**Appendix Table 4. Aliphatic secondary amines and volatile *N*-nitrosamines in tobacco and tobacco smoke**

| $R_1$-NH-$R_2$ | | | Identified in tobacco (T) or smoke (S) | | | | Identified in tobacco (T) or smoke (S) | | |
|---|---|---|---|---|---|---|---|---|---|
| $R_1$ = | $R_2$ = | CAS no. | T | S | *N*-Nitrosamine | CAS no. | T | S | Activity |
| $CH_3$- | $CH_3$- | 124-40-3 | x | x | ***N*-Nitrosodimethylamine** [a] | 62-75-9 | x | x | (+) [1] [b] |
| $CH_3$- | $CH_3CH_2$- | 624-78-2 | x | x | ***N*-Nitrosoethylmethylamine** | 10595-95-6 | x | x | (+) [52] [b] |
| $CH_3$- | $CH_3(CH_2)_2$- | 627-35-0 | x | — | *N*-Nitrosomethylpropylamine | 924-46-9 | — | x | (+) [66] [b] |
| $CH_3$- | $(CH_3)_2CH$- | 4747-21-1 | x | x | *N*-Nitrosoisopropylmethylamine | 34419-76-6 | x | x | |
| $CH_3$- | $CH_3(CH_2)_3$- | 110-68-9 | x | x | ***N*-Nitrosobutylmethylamine** | 7068-83-9 | — | x | (+) [71] [b] |
| $CH_3$- | $(CH_3)_2CHCH_2$- | 2504-18-9 | — | — | *N*-Nitrosoisobutylmethylamine | | — | x | |
| $CH_3$- | $(CH_3)(C_2H_5)CH$- | | — | x | *N*-Nitroso-*sec*-butylmethylamine | | — | — | |
| $CH_3$- | $(CH_3)_2CH(CH_2)_2$- | | — | x | *N*-Nitrosoisopentylmethylamine | | — | — | |
| $CH_3$- | $CH_2=C(CH_3)$- | 22023-64-9 | — | x | *N*-Nitrosoisopropylidenemethylamine | | — | — | |
| $CH_2CH_3$- | $CH_2CH_3$- | 109-89-7 | x | x | ***N*-Nitrosodiethylamine** | 55-18-5 | — | x | (+) [7] [b] |
| $CH_2CH_3$- | $CH_3(CH_2)_2$- | 20193-20-8 | — | — | *N*-Nitrosoethylpropylamine | 25413-61-0 | — | x | |
| $CH_2CH_3$- | $(CH_3)_2CHCH_2$- | | — | — | *N*-Nitrosoethylisobutylamine | 71607-99-3 | — | x | |
| $CH_2CH_3$- | $CH_3(CH_2)_3$- | 13360-63-9 | — | — | *N*-Nitroso-*n*-butylethylamine | | — | x | (+) [122] [b] |
| $CH_3(CH_2)_2$- | $CH_3(CH_2)_2$- | 142-84-7 | x | x | ***N*-Nitrosodi-*n*-propylamine** | 621-64-7 | — | x | (+) [21] [b] |
| $CH_3(CH_2)_2$- | $(CH_3)_2CH$- | 21968-17-2 | x | x | *N*-Nitrosoisopropylpropylamine | | — | — | |
| $CH_3(CH_2)_2$- | $(CH_3)(C_2H_5)CH$- | | — | x | *N*-Nitroso-*sec*-butylpropylamine | | — | — | |
| $(CH_3)_2CH$- | $(CH_3)_2CH$- | 108-18-9 | — | x | *N*-Nitrosodiisopropylamine | 601-77-4 | — | — | (+) [34] [b] |
| $(CH_3)_2CH$- | $CH_3(CH_2)_3$- | 39099-23-5 | — | x | *N*-Nitrosobutylisopropylamine | | — | — | |
| $CH_3(CH_2)_3$- | $CH_3(CH_2)_3$- | 111-92-2 | x | — | *N*-Nitrosodi-*n*-butylamine | 924-16-3 | x | x | (+) [36] [b] |
| $CH_3(CH_2)_3$- | $(CH_3)_2CHCH_2$- | 20810-06-4 | — | x | *N*-Nitrosobutylisobutylamine | | — | — | |
| $(CH_3)(C_2H_5)CH$- | $(CH_3)(C_2H_5)CH$- | 626-23-3 | x | — | *N*-Nitrosodi-*sec*-butylamine | | — | — | (+) [45] [b] |
| $(CH_3)_3C$- | $(CH_3)_2CH$- | | — | x | *N*-Nitroso-*tert*-butylisopropylamine | | — | — | |

[a] Compounds displayed in bold are listed as toxicants in Table 1 of the main part of our report.
[b] Bioassay results in laboratory animals are summarized in Preussmann and Stewart (57). (+) indicates tumor induction, (–) indicates negative response. Number in [ ] is catalog number in Preussmann and Stewart (57).




**Appendix Figure 2. An aromatic amine (III), pyrrolidine (IV), piperazine (V), piperidine (VI), 1,2,3,6-tetrahydropyridine (VII)**




**Appendix Figure 3. Indole (VIII), carbazole (IX), and 1*H*-benzimidazole (X)**

In Appendix Table 5 are listed 32 secondary amines, most of which have been identified in tobacco and/or smoke. In only a few cases have the corresponding NNAs been identified as tobacco and/or smoke components. It is highly probable that the NNAs corresponding to the remaining secondary amines are also tobacco smoke components.

Among the numerous classes of smoke components are several other types of secondary amines, e.g., the pyrroles, indoles, carbazoles, imidazoles. However, their highly aromatic nature and the acidity of the imino hydrogen probably preclude any significant *N*-nitrosation either in the tobacco or during the smoking process. A dozen or so substituted pyrroles; nearly 50 alkyl derivatives of indole (**VIII**); carbazole (**IX**) and several of its alkyl derivatives, benzocarbazoles, and dibenzocarbazoles; and several alkyl derivatives of imidazole and benzimidazole (**X**) have been reported as tobacco smoke components. Even though each of them could theoretically yield an NNA, no NNA corresponding to any of them has been identified to date in tobacco smoke (Appendix Figure 3).

It is obvious that the number of NNAs in tobacco/tobacco smoke might be substantially greater than the 40 or so NNAs now known to be present. Since the per cigarette yields of the yet unidentified NNAs may be at the picogram or femtogram levels, their contribution to MSS toxicological properties may not be particularly meaningful or important. However, they may be just as important from a biological point of view as those MSS components repeatedly listed as toxicants for which no or questionable quantitative data are available.

To put the NNAs in perspective and to determine how many more are actually present in MSS, what may be needed is an extensive study corresponding to the PAH (9,13,14) and aza-arene (15) studies conducted in the 1970s.

It is also interesting to note that 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), the major metabolite of NNK (12), is usually not listed as a cigarette MSS toxicant even though NNAL has been reported to be both tumorigenic to several rodent species (56) and mutagenic in the Ames *Salmonella typhimurium* test.

While the MSS toxicants listed in Table 1 in the main body of our report number about 150, their number could be increased in future published lists (58) by inclusion of the individual dioxins plus components in Appendix Tables 1, 4, and 5. The increase in number would far outweigh the decrease resulting from deletion of the problematic components we discussed earlier.

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**Appendix Table 5. Alicyclic and aromatic secondary amines and *N*-nitrosamines in tobacco and tobacco smoke**

| Amine | CAS no. | Identified in tobacco (T) or smoke (S) T | S | *N*-Nitrosamine | CAS no. | Identified in tobacco (T) or smoke (S) T | S | Activity |
|---|---|---|---|---|---|---|---|---|
| *Aromatic amine (**III**)* $R_1$-NH-$R_2$ | | | | | | | | |
| $R_1$ = $R_2$ = | | | | | | | | |
| $CH_3$- $C_6H_5$- | 100-61-8 | — | x | *N*-Nitroso-*N*-methylphenylamine | 614-00-6 | — | — | (+) [108] [a] |
| $CH_3$- 2-$CH_3$-$C_6H_4$- | 611-21-2 | — | x | *N*-Nitroso-*N*-methyl-2-toluidine | | — | — | |
| $CH_3$- 4-$CH_3$-$C_6H_4$- | 623-08-5 | — | x | *N*-Nitroso-*N*-methyl-4-toluidine | | — | — | |
| $CH_3$- 2-$C_2H_5$-$C_6H_4$- | 1821-38-1 | — | x | *N*-Nitroso-2-ethyl-*N*-methylaniline | | — | — | |
| $CH_3$- 3-$C_2H_5$-$C_6H_4$- | 71265-20-8 | — | x | *N*-Nitroso-3-ethyl-*N*-methylaniline | | — | — | |
| $CH_3$- 4-$C_2H_5$-$C_6H_4$- | 37846-06-3 | — | x | *N*-Nitroso-4-ethyl-*N*-methylaniline | | — | — | |
| $CH_3$- $C_6H_5$-$(CH_2)_2$- | 589-08-2 | — | x | *N*-Nitroso-*N*-methylphenylethylamine | | — | — | |
| $CH_3$- 4-$NH_2$-$C_6H_4$- | | — | x | *N*-Nitroso-*N*-methyl-4-aminoaniline | | — | — | |
| $C_2H_5$- $C_6H_5$- | 103-69-5 | — | x | *N*-Nitroso-*N*-ethylaniline | 612-64-6 | — | — | |
| $C_2H_5$- 2-$CH_3$-$C_6H_4$- | 94-68-8 | — | x | *N*-Nitroso-*N*-ethyl-2-toluidine | | — | — | |
| $C_6H_5$- $C_6H_5$- | 122-39-5 | — | x | *N*-Nitrosodiphenylamine | 86-30-6 | — | — | (–) [55] [a] |
| $C_6H_5$- 4-$(CH_3)_2$CH-$C_6H_4$- | 5650-10-2 | — | x | 4-Isopropyl-*N*-nitrosodiphenylamine | | — | — | |
| *Pyrrolidine (**IV**)* | 123-75-1 | — | x | ***N*-nitrosopyrrolidine** | 930-55-2 | — | x | (+) [146] [a] |
| 2-$CH_3$- | 765-38-8 | x | x | *N*-Nitroso-2-methylpyrrolidine | | x | — | |
| 3-$CH_3$- | 34375-89-8 | — | x | *N*-Nitroso-3-methylpyrrolidine | | — | — | |
| 2,4-di$CH_3$- | 13603-04-8 | — | x | *N*-Nitroso-2,4-dimethylpyrrolidine | | — | — | |
| 2,5-di$CH_3$- | 3378-71-0 | — | x | *N*-Nitroso-2,5-dimethylpyrrolidine | 55556-86-0 | — | — | (+) [148] [a] |
| 2-$CH_3CH_2$- | 1003-28-7 | — | x | *N*-Nitroso-2-ethylpyrrolidine | | — | — | |
| 2-keto- | 616-45-5 | x | — | *N*-Nitroso-2-pyrrolidone | | — | — | (–) [301] [a] |
| *Piperazine (**V**)* | 110-85-0 | — | — | *N*-Nitrosopiperazine | 5632-47-3 | — | — | (+) [200] [a] |
| | | | | *N,N'*-Dinitrosopiperazine | 140-79-4 | — | — | (+) [207] [a] |
| 1-$CH_3$- | 109-01-3 | — | x | *N*-Nitroso-*N'*-methylpiperazine | 16339-07-4 | — | — | (+) [201] [a] |
| 2-$CH_3$- | 109-07-9 | — | x | *N,N'*-Dinitroso-2-methylpiperazine | 55556-94-0 | — | — | (+) [208] [a] |
| 2,5-di$CH_3$- | 106-55-8 | — | x | *N,N'*-Dinitroso-2,5-dimethylpiperazine | 55556-88-2 | — | — | (+) [209] [a] |
| *Piperidine (**VI**)* | 110-89-4 | — | x | ***N*–Nitrosopiperidine** [b] | 100-75-4 | | x | (+) [160] [a] |
| 2-$CH_3$- | 109-05-7 | — | x | *N*-Nitroso-2-methylpiperidine | 7247-89-4 | — | — | (+) [175,176] [a] |
| 3-$CH_3$- | 626-56-2 | — | x | *N*-Nitroso-3-methylpiperidine | 13603-07-1 | — | — | (+) [177] [a] |
| 2-COOH | 535-75-1 | x | — | *N*-Nitroso-2-piperidinecarboxylic acid | 4515-18-8 | x | — | (–) [172] [a] |
| 3-COOH | | — | — | *N*-Nitroso-3-piperidinecarboxylic acid | 65445-62-7 | x | — | |
| 4-COOH | | — | — | *N*-Nitroso-4-piperidinecarboxylic acid | 6238-69-3 | x | — | (–) 173] [a] |
| 2,3-di$CH_3$- | 23513-39-5 | — | x | *N*-Nitroso-2,3-dimethylpiperidine | | — | — | |
| 2,4-di$CH_3$- | 6287-19-0 | — | x | *N*-Nitroso-2,4-dimethylpiperidine | | — | — | |
| 2,6-di$CH_3$- | 504-03-0 | — | x | *N*-Nitroso-2,6-dimethylpiperidine | 17721-95-8 | — | — | (–) [180] [a] |
| 2-$C_2H_5$- | 1484-80-6 | — | x | *N*-Nitroso-2-ethylpiperidine | | — | — | |
| 2-$(CH_3)_2$CH- | | — | x | *N*-Nitroso-2-isopropylpiperidine | | — | — | |
| 4-keto- | | — | x | *N*-Nitroso-4-piperidone | 55556-91-7 | — | — | (+) [163] [a] |
| *Pyridine, 1,2,3,6-tetrahydro- (**VII**)* | 694-05-3 | — | x | *N*-Nitroso-1,2,3,6-tetrahydropyridine | 55556-92-8 | — | — | (+) [168] [a] |

[a] Bioassay results in laboratory animals are summarized in Preussmann and Stewart (57). (+) indicates tumor induction, (-) indicates negative response. Number in [ ] is catalog number in (57).

[b] Compounds displayed in bold are listed as toxicants in Table 1 of the main part of our report.


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