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Chemistry of cigarette smoking – A review

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This comprehensive article illustrates the facts about cigarette smoking, dealing primarily with the underlying chemistry and biochemical features linked to tobacco related effects. A major attempt is made to discuss crucial factors concerning the chemical effects of tobacco smoke. In that regard following aspects are incorporated into the discussion: The chemical contents of original tobacco and its pyrolytic products in the smoke; The pyrolysis; The physico-chemical nature of nicotine molecule: biosynthesis, metabolism and receptor interaction; The nicotine addiction; The harmful effects of tobacco and other thermolytic chemicals on health. The detection of smoke inhalation by using suitable bio-chemical markers. Additional discussion is provided regarding the importance of pH during tobacco processing, which plays a distinctive role on nicotine absorption and influencing the addiction. The use of nicotine patch, gum or lozenges to avoid the harmful effects of smoke are also reviewed. More deliberation is offered on the action of nicotine with regard to biosynthesis, metabolism, receptor interaction and its physiologic role, considering the molecule to be an important item of tobacco, enabling to provoke addiction in the brain. The usefulness of synthetic mimicry of nicotine is also brought into light for its preventive role on addiction as well as to treat Alzheimer’s and Parkinson’s or other neurological diseases. Additionally, cotinine, the major metabolite of nicotine is also momentarily reviewed in the light of similar medicinal approaches. In brief, the review describes the overall chemistry and biochemistry involved in the use of tobacco.

**Keywords**: Tobacco, nicotine, cotinine, α-naphthylamine, 4-aminobiphenyl, heterocyclics, nAChR, benzo[a]pyrene, benzene, dopamine, NRT, LTP, cytochrome P450-2A6, VTA, Ca$^{2+}$-channel, pyrolysis, Alzheimer’s disease, Parkinson’s disease

The smoking or chewing of tobacco leaves is practiced for several millennia. In every continent and society irrespective of the cultural differences, the use of tobacco is a common habit. Although consumed mainly to enjoy the leisure moments, but its persistent use through smoking or chewing builds up a habit that results in serious addiction. Aside the addictive nature, tobacco also imposes serious health risks which is unanimously confirmed by the major scientific studies. The physician communities around the world vehemently oppose its uses in any form. It is established that chemicals generated during cigarette burning are seriously hazardous to the health. Admittedly, smoking tobaccos may not manifest in any wrongful behavior or inflicts immediate death as does often occur in the case of street drugs like cocaine, heroin and others. But despite the social acceptance and no imminent dangers involved, its perilous effects on health are evident only after the continued use over a long period of time which is an established fact. But even with consistent spreading of the awareness against its uses, this horrid practice continues almost unabated. Indeed, it offers temporary relaxation that helps recover the confidence while pursuing any stressful works, which in reality compels a person to search for more, making it a regular habit. In that way, the indulgence to prolong consumption provokes the addiction. The chewing of tobacco as an alternative although seems less hazardous but is not conducive to the health either. This practice creates serious health problems also in the long run.
Thus, considering the health issues, physicians anywhere in the world recommend staying away from the use of tobacco\(^7\).

What is tobacco? It is the leaf from a plant belonging to *Solanaceae* family, also known as *Nicotania*\(^8\). The leaf contains chlorophyll, water, various sugars, minerals, alkaloids and polyphenols. The number of alkaloids found in tobacco leaves are \(\sim 20\). Among those, six of them are the prominent ones of which nicotine exists at the highest level \(\sim 25 \text{ mg/g}\). The others are nornicotine \(\sim 0.2 \text{ mg/g}\), neo-nicotine \(\sim 0.09 \text{ mg/g}\), anatabine \(\sim 0.6 \text{ mg/g}\) and myosmine \(\sim 0.1 \text{ mg/g}\). The major classes of poly-phenols found are Rutin \(\sim 186 \mu\text{g/g}\), Quercitin \(\sim 156 \mu\text{g/g}\) and Scopoletin \(\sim 0.1 \text{ mg/g}\) (Figure 1)\(^9\). In addition, there are a few isoprenoids and carotenoids found in the tobacco leaves which have distinctive roles in providing the flavors, like Linalool or Geraniol, *etc.* (Figure 1)\(^9\). But the quality of tobacco imparting longer satisfying effect depends largely on the nicotine content. Nicotine offers both pleasure and reward sensation inside the brain instigating the dependency or addiction\(^10\). In the early days immediately after discovery, the leaves were also used to treat unidentified diseases due to its alkaloid content. Recently, nicotine has been identified to have positive roles in controlling Alzheimer’s and Parkinson’s disorders\(^11\). But one has to keep in mind, that it has far more adverse effects on health. Therefore the use of tobaccos is restricted and campaign against its use should be kept intense.

The report provided by CDC (Atlanta, GA, USA) and US Surgeon General’s Office (Washington D.C.) indicates that the overall death in the last fifty years due to cigarette smoking and tobacco related causes crosses more than 20 million. The report also describes that higher incidences of innumerable deadly diseases of colon, lungs, prostate, bladder and heart arises owing to the smoking including also several horrid illnesses like arthritis, blindness and many more\(^9\). So far, the cost of burden accumulates to \(\sim \$300\) billion which eventually would be soaring in the future. Approximately, six million children would die prematurely before reaching the age of eighteen due to the smoking related illnesses\(^9,10\). Among the chronic diseases created by smoking, the most virulent ones are the cancers of numerous organs (Lung, Colon, Prostate, [Figure 1 — Major tobacco ingredients](#)
American Lung Association displays that about 600 toxic chemicals present in an unburnt cigarette produce 7000 hazardous products while undergoing burning. The most serious ones are, acetone, acetic acid, ammonia, arsenic, benzene, several aromatics, butane, cadmium, carbon monoxide, formaldehyde, hexamine, lead, naphthalene, tricyclic aromatics, menthol, nicotine, tar, toluene, etc. A large section of them are cumulative by nature, so continuous practice of smoking allows these to accumulate inside the body imposing severe harm in the future. The nature of their toxicity is well known. About 43 of them are characterized as cancer causing agents 9,12. Nicotine, the main ingredient, is not defined as carcinogenic according to the CDC but it has an addictive quality that adversely affects many physiologic parameters 12,13. Adding the addictive role of nicotine along with the toxic products generated during smoking raises a major concern about the habit of tobacco use.

**Major ingredients**

It is determined that the basic ingredients of tobaccos are carbohydrates (~ 75%), proteins (~ 7 – 10%) and alkaloids which includes nicotine and its derivatives (30 – 50 mg/g) along with terpenes and polyphenols in lesser degrees 14. As mentioned earlier, the terpenes and polyphenols provide the aroma whereas the nature of carbohydrates determine the quality regarding moisture contents, smoothness, etc. All these factors depend predominantly on the place of origin and heredity. Chemically, the carbohydrates found in tobaccos are broadly classified as: (a) Starch and sugars, (b) Sugar esters, (c) Glucosides, (d) Cellulose, (e) Hemicellulose and (f) Pectin.

**Starch and sugars:** Starch is a polymer of glucose linked by the glycosidic bond. Its content varies with the aging and also during curing of the tobacco leaves, for example ~ 25% in green, ~ 12% in yellow, ~ 6% in the cured state whereas the amount of reducing sugars follows backward mode; ~ 7% in green, 16% in yellow and 17% in the cured phase. The similar trend holds for the sucrose content also, ~ 1.8% in green, 5.2% yellow and 7.5% in the cured condition. These variations are caused due to the arrested bio-respiration at different stages of the leaves allowing breaking down of the starch molecules producing more reducing sugars and sucrose. In that process, surrounding pH stays almost the same 14.

**Sugar esters:** They are mainly sucrose or glucose tetra-esters of lower carboxylic acids. They provide aroma during thermolysis subsequently releasing also the free fatty acids. Interestingly, totally esterified sucrose or glucose esters like glucose pentaisovalerate or sucrose octaesters do not undergo thermolysis easily to release the acid groups. Both the sugars and its esters add flavors to the smoke, enhancing nicotine’s addictive performance in the brain 15.

**Glucosides:** Terpenoids and polyphenols are the aroma providers linked with mono or disaccharides molecules produced by the oxidative transformation of various carotenoid pigments. They are released as a result of natural hydrolysis of the glucosides. The prime example is 3-hydroxy-β- damascone. The aromatic flavor is also enhanced by frequent treatment with the enzymes from outside in addition to natural autolytic procedure. Often, this hydrolysis is carried out commercially to add the extra flavors. The isolation of aglucone by hydrolysis from the leftovers of unused leaves is routinely performed in order to add extra aroma to the cigarettes or pipe tobaccos 16.
Cellulose: Usually, most of the tobaccos contain ~10% of cellulose and hemicellulose. As per chemical nature, cellulose is a polysaccharide and thus bears a general formula of \((\text{C}_6\text{H}_{10}\text{O}_5)_n\) and consists of many covalently linked glucose units. Its hydrolysis from tobacco leaves yields glucose as well as galactose units also. A high cellulose content could lower the quality of tobaccos. The degree of polymerization often stays within about 1100-1600. Its pyrolysis mainly produces formic acid (HCOOH) or formaldehyde (HCHO) which are identified in the cigarette smoke.

Hemicellulose: Unlike the cellulose, hemicellulose is a hetero-polymer of various sugar units found on the plant cell walls associated with lignin (high molecular weight polyphenolic product). Whether it is covalently linked or physically trapped with the lignin remains unclear. In tobacco leaves, partial hydrolysis often produces glucose, arabinose, and galactose and to a lesser extent, xylose which always varies according to its place of origin or genetic lineage. It adds smoothness to the tobaccos by holding the moisture content. Like cellulose, its pyrolysis also produces formaldehyde and formic acid.

Pectin: Tobacco leaves possess 6-12% of its weight as pectin. It is a carbohydrate polymer of 1,4 linked O-galactouronic acids having varying stages of methylation. Within the poly-galactouronic acid backbones there exists neutral sugar molecules like galactose, arabinose and rhamnose, etc. Pectin acts as a glue holding the other molecular objects within the matrix of the leaves. Its pyrolysis at around 200-310°C creates both formic and acetic acids. It basically determines the moisture content of the tobaccos.

Acidity generated by the degradation of carbohydrates and its subsequent interaction with \(\text{NH}_3\) produces significant impact on the character of tobaccos. Its cumulative effects determines the water pH within each puff. The brands having less sugars create alkaline smoke due to higher \(\text{NH}_3\) content. \(\text{NH}_3\) is produced from the nitrogenous substances of the leaves at the temperature range of 190-300°C. The high level of \(\text{NH}_3\) or less sugar content affect the ratio of free nicotine / protonated nicotine – salts produced in the smoke. The high smoke pH having more free nicotine affects the sensory impression. It helps its absorption along with the other alkaloids.

Nicotine exists in nature as levorotary having \(S\) & \(R\) isoforms. The \(S\)-enantiomer occurs about ~99% whereas \(R\)-isomer exists only ~0.2% of the total nicotine content. The actual nicotine level in cigarettes is around ~6.2 - 13.0 mg/cig which is ~1.23 ± 0.15% of the tobacco weight. For imported ones, the levels are somewhat higher, ~7.2-28.9 mg/cigarette or 1.80±0.25%. The level is high in the case of pipe tobaccos which is 30.1-50.9 mg for normal quantity held in a pipe or ~3.8% of the tobacco weight. Smoking a cigarette introduces ~0.1- 0.5 mg of nicotine in the body, so the circulating concentration reaches to \(≥\,124\times10^{-9}\text{M/L}\).
During smoking, nicotine enters into circulation through inhalation via lung and finally reaches the brain within ~ 7-10 seconds causing stimulated release of several neurotransmitters like acetylcholine, nor-epinephrine, epinephrine, serotonin, dopamine, β-endorphin, arginine-vasopressin, etc. The triggered release of acetylcholine increases mind’s focus and also the memory. In combination with nor-epinephrine it augments the alertness level. The release of nor-epinephrine occasionally induces sexual excitement. The high level of acetylcholine and β-endorphin together lowers the pain sensation while β-endorphin alone reduces the anxiety level. The behavioral aspect of nicotine relies on dopamine which enhances the reward systems. Considering all those features, especially the rewarding event, habit of smoking enables to incite the addictiveness.

Identifiably, the most harmful effect of smoking is created by the production of tars during the pyrolysis of tobacco. The average tar content is ~ 21.7 mg/cigarette. It carries many undesirable chemicals of which some are considered to be carcinogenic (Table I). The recent regulation by FDA (Jan 1/2004) restricts the tar content limiting to 10 mg/cigarette. The native tobacco leaves does not possess any tar ingredients. Among the potent carcinogens produced during pyrolysis are benzo[a]pyrene (20-40 ng/cig) and α-NA (171-180 ng/cig) and 4-ABP (~ 100 ng/cig). They are considered to be the most virulently carcinogenic. There are other polyaromatic hydrocarbons (PAH) produced during the pyrolysis / pyrosynthetic reactions of long chain hydrocarbons, terpenes, phytosterols (stigmasterol), paraffins, sugars, aminoacids and celluloses present in the unburnt tobaccos. Additionally, the wrapping papers which are prepared from cellulose and are impregnated with the additives also produce harmful benzopyrene on pyrolysis. Further, the nitrosamines generated in the process are identified to be potent carcinogens. Chemically, nitrosamines are the organic compounds bearing -N-N=O group. They are produced by covalent linkage of -N=O to the amines in tobaccos. Initially -NO2 is generated from the -NO3. 

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount (µg/Cig)</th>
<th>Compounds</th>
<th>Amount (µg/Cig)</th>
<th>Compounds</th>
<th>Amount (µg/Cig)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine</td>
<td>1000 – 3000</td>
<td>Bipyridils</td>
<td>10.0 – 30.0</td>
<td>Phenol</td>
<td>80 – 160</td>
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<tr>
<td>Nornicotine</td>
<td>5 – 150</td>
<td>n-Hentriacontane</td>
<td>100</td>
<td>Other phenols</td>
<td>60 – 180</td>
</tr>
<tr>
<td>Anatabine</td>
<td>5.0 – 15</td>
<td>Non Volatile HC</td>
<td>300 – 400</td>
<td>Catechol</td>
<td>200 – 400</td>
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<tr>
<td>Anabasine</td>
<td>5.0 – 12</td>
<td>Naphthalene</td>
<td>2.0 – 4.0</td>
<td>Other Catechols</td>
<td>100 – 200</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>20 – 50</td>
<td>Naphthalenes</td>
<td>3.0 – 6.0</td>
<td>Other Dihydroxy benzenes</td>
<td>200 – 400</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>100 – 200</td>
<td>Anthracenes</td>
<td>0.05 – 0.1</td>
<td>Scopoletin</td>
<td>15 – 30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fluorenes</td>
<td>0.6 – 1.0</td>
<td>Cyclotenes</td>
<td>40 – 70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pyrenes</td>
<td>0.3 – 0.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fluoranthenes</td>
<td>0.3 – 0.45</td>
<td>Solanesol</td>
<td>600 – 1000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carcinogen PAH</td>
<td>0.1 – 0.25</td>
<td>Neophytiadines</td>
<td>200 – 350</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Limonene</td>
<td>100 – 150</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Palmitic acid</td>
<td>100 – 150</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Steraic acid</td>
<td>50 – 75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Oleic acid</td>
<td>4 – 110</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Linoleic acid</td>
<td>150 – 250</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Linolenic acid</td>
<td>150 – 250</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lactic acid</td>
<td>60 – 80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Indole</td>
<td>10.0 – 15.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Skatole</td>
<td>12.0 – 16.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Quinolines</td>
<td>2.0 – 4.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Benzofuranes</td>
<td>200 – 300</td>
</tr>
</tbody>
</table>

Table I—Organic constituents of mainstream smoke.
Figure 2 — Nitrosation reactions between –NO₂ group and members of the nicotine family.²⁴
Table II — Carcinogenic hydrocarbons and heterocyclics in cigarettes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Aza Arenes</th>
<th>N- Nitroso Amines</th>
<th>Aromatic Amines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benz (a) anthracene</td>
<td>Quinoline</td>
<td>N-nitrosodimethylamine</td>
<td>2-Toluidine</td>
</tr>
<tr>
<td>Benzo (b) fluoranthene</td>
<td>Dibenzo(a,h) acridine</td>
<td>N-nitrosodiethylamine</td>
<td>2-Naphthylamine</td>
</tr>
<tr>
<td>Benzo (k) fluoranthine</td>
<td>Dibenzo(a,j) acridine</td>
<td>N-nitrosoethylmethylamine</td>
<td>4-Aminobiphenyl</td>
</tr>
<tr>
<td>Benzo (a) pyrene</td>
<td>7H-Dibenzo(c,g)-carbazole.</td>
<td>N-nitrosopyrrolidone</td>
<td></td>
</tr>
<tr>
<td>Dibenzo (a,h) anthracene</td>
<td></td>
<td>N-nitrosodihexamolene</td>
<td></td>
</tr>
<tr>
<td>Dibenzo (a,i) pyrene</td>
<td></td>
<td>N-nitrosoaracine</td>
<td></td>
</tr>
<tr>
<td>Dibenzo (a,l) pyrene</td>
<td></td>
<td>N-nitrosornicotine</td>
<td></td>
</tr>
<tr>
<td>Indeno (1,2,3-cd) pyrene</td>
<td></td>
<td>4-(Methyl-2-nitrosoacetamido)-4-(pyridyl)-1-butanol</td>
<td></td>
</tr>
<tr>
<td>5-Methyl chrysene</td>
<td></td>
<td>N'-nitrosoanabase</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N'-nitrosomorpholine</td>
<td></td>
</tr>
</tbody>
</table>

derivatives present in tobaccos, produced by the bacterial enzymes during fermentation and curing\textsuperscript{20}. The -NO\textsubscript{2} conjugates with all the amines causing nitrosation (Figure 2). So the amino acids and other amines present like nicotine, nor-nicotine, anabasine and anatabine also undergo the nitrosation process\textsuperscript{26-28}. Pyrolysis also creates CO in significant amounts (10 mg/cig effluent) which is considered the most harmful agent\textsuperscript{26}. Lowering of tar content decreases the CO level in smoke. Besides, the low level of other poisonous gases like HCN (~ 450 µg/cig), H\textsubscript{2}S (~ 60 µg/cig) and various oxides of nitrogen (~ 500 µg/cig) are also detected. Table II lists the major harmful ingredients present in the mainstream smoke of an unfiltered cigarette of which nicotine exists at the highest level\textsuperscript{9,26}.

Further, the pesticides used during tobacco cultivation are also a factor of much concern. It is stated by the US Surgeon General’s report that they exist in the prepared tobaccos in considerable amounts thereby imposing health risks to the smokers\textsuperscript{26,27}. In general, numerous pesticides, which are recognizably hazardous to the health of smokers, are used for maintaining the healthy plants. Even after careful and thorough processing, a substantial level of pesticides are retained in the final products. The most used ones are organochlorine compounds and they are detected at the following concentrations: α-Endosulfan (22 µg/Kg), β-Endosulfan (21 µg/Kg), Heptachlor-Epoxide (47 µg/Kg), 4,4 Dicloro-diphenyl-dichloro ethane or DDE (17 µg/Kg)\textsuperscript{28,29}. The Endosulfans are known to be neurotoxic and endocrine disruptors. Endosulfan is an antagonist to the GABA gated Cl\textsuperscript{-} channel and in that way acts as an inhibitor to the Ca\textsuperscript{2+} and Mg\textsuperscript{2+} ATPase. It is a xenoestrogen that enhances the estrogen action imposing high risks to health with a large possibility of birth defects. They are also proven carcinogens\textsuperscript{29-31}.

![Figure 3 — Temperature differential of cigarette burning – Combustion zone and Pyrolysis / Distillation zone. Combustion zone → O\textsubscript{2} reacts to form simple gases CO and CO\textsubscript{2} at 700-900°C. Pyrolysis / Distillation zone → O\textsubscript{2} levels are low, 1/3 of the tobacco constituents are distilled out. Low MW hydrocarbons stays in the vapor phase. n-Alkanes, naphthalenes are produced at ~ 700°C in partial burning zone are in the particulate phase. At ~ 500°C benzene, alkyl-benzene and about 75 aromatics, sugars, amino acids, fatty acids, ring compounds are generated and transformed to particulate phase](image)

**Chemical processes involved in cigarette burning**

Cigarette burning follows complex physicochemical processes. Usually two regions are defined inside the burning zone: (a) combustion zone and (b) pyrolysis or distillation zone (Figure 3). The temperature within the combustion zone ranges between 700 to 950°C (glowing area) when drawing the air during puffing. The O\textsubscript{2} from air flow initiates carbonization producing CO and CO\textsubscript{2}. Downstream of that zone is the pyrolysis or distillation zone.
Its temperature ranges from 600 to 200°C where the extent of free O$_2$ drops to a relatively low level. At this stage, ~ 1/3 of the constituents including nicotine and the others distill out. The smoke that comes out consists of super saturated vapor which is cooled to an ambient temperature within a few milliseconds and simultaneously condenses into the aerosol particles.

Physically, smoke is a stable aerosol composed of heterogeneous mixture of gases, vapors, liquid and particles. It comprises of ~ 1.0 million particles / cubic centimeter whose median size is ~ 0.5µ. During pyrolysis the average temperature within burning zone soars to ~ 884°C. A large number of chemical reactions then sets up at this temperature involving oxidation, dehydrogenation, cracking, rearrangement and later, the condensation. Interestingly, some ingredients can still distill out unchanged. The low molecular weight hydrocarbons that are normally produced within the pyrolysis zone stay at the vapor phase of smoke whereas the n-alkanes and n-alkenes generated at relatively higher temperature (700°C) are drawn out as particulate matter. Around 500°C, benzene and alkyl benzenes are formed. More than 75 monocyclic aromatics like benzene and others are produced from the pyrolysis of amino acids, fatty acids, cinnamic acids, sugars, and paraffins. At temperatures over 700°C, naphthalenes are generated. About 80 naphthalene products are detected in the smoke. The mixture containing these obnoxious agents is termed as tar. But this is not a very well defined phase due to varying level of the ingredients.

In experimental terms, tar is defined as being those ingredients that are residing within the particulate phase minus water and nicotine, which can be collected on the Cambridge filter pad in a smoking machine while coming out of the mainstream smoke. The smoke components are distributed in particulate and vapor phase in the aerosol. The Cambridge filter is used in detecting smoke as part of an analytical procedure. It is made of glass filters having organic binders (Cambridge Filter Corp. Syracuse, NY). It has 99.99% efficiency to trap the aerosol particles of > 0.1µm diameter. Normally, substances below MW ~ 60 stay in the vapor phase whereas those over MW ~ 200 are in the particulate phase. The carcinogenic hydrocarbons, aromatics and heterocyclics are mainly in the particulate phase.

### Additives:

Historically, additives are introduced into the tobacco to enhance its taste, smell or to satisfy any sensory aspects. Frequently, cocoa and sugars are added to boost the aroma and flavor. Often, menthol is also added to create a special taste. Following are the underlying reasons behind the use of additives. Table III shows a list of major additives normally used during the manufacture of cigarettes. Their use is intended for the following reasons.

- Humectants (sugars, glycerol and glycol like compounds) are added to keep the smoke moist.
- Flavors of different nature to create special brands (Vanillin, Limonene, Cedrol, Furaneol, etc).
- Menthol, sweeteners to provide smoothness for easy inhalation.
- Some additives are designed for the reduction of second hand smoke [Al$_2$(SO$_4$)$_3$, NaH$_2$PO$_4$].
- Some additives are used to make the cigarette more appealing (Levulinic acid).
- Some pharmacologically active compounds are used to enhance the addiction (Bronchodilators, extra quantities of added nicotine).

The most common agent added in preparing the tobaccos is NH$_3$ or any of its derivatives, like (NH$_4$)$_2$HPO$_4$, NH$_4$HCO$_3$, NH$_2$OH or (NH$_2$)$_2$C=O to enhance the nicotine absorption for provoking addiction. The FDA often argues that it is a deliberate act whereas according to the manufacturers’ view, it is a traditional practice carried over from the past, before their role in enhancing the nicotine addiction was even known.

<table>
<thead>
<tr>
<th>Additives</th>
<th>Use</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>Tobacco</td>
<td>Humectant</td>
</tr>
<tr>
<td>Hexanal</td>
<td>Tobacco</td>
<td>Flavor</td>
</tr>
<tr>
<td>Lemon Oil</td>
<td>Tobacco</td>
<td>Flavor</td>
</tr>
<tr>
<td>Linalool</td>
<td>Tobacco</td>
<td>Flavor</td>
</tr>
<tr>
<td>Potassium sorbate</td>
<td>Tobacco</td>
<td>Preservative</td>
</tr>
<tr>
<td>Propyl-PHB</td>
<td>Tobacco</td>
<td>Preservative</td>
</tr>
<tr>
<td>Sugars – Invert sugars</td>
<td>Tobacco</td>
<td>Humectant &amp; Flavor</td>
</tr>
<tr>
<td>Cocoa &amp; Theobromine</td>
<td>Tobacco</td>
<td>Enhances expansion of airways and facilitates smoke increase and intake of nicotine.</td>
</tr>
<tr>
<td>Glycyrrhizin</td>
<td>Tobacco</td>
<td>Liquorice ingredient and acts as bronchodilator</td>
</tr>
</tbody>
</table>
Figure 4 — Deprotonation of nicotine molecule by NH₃

The FDA indicates that the intent of NH₃ use is to increase the delivery of free base nicotine which would augment the addictions. The original tobacco leaves are acidic by nature, ~pH 3.0. Treating with NH₃ or its salts makes available the free nicotine base due to increase of pH. The average concentration of NH₃ detected in commercial tobacco of this country is 0.1-0.4%. It is released in both gas and particulate form during smoking. The smoke pH varies widely from blend to blend. For American blends, the pH ranges between 5.8-6.2, which is slightly acidic. So nicotine molecules are in the mono-protonated form in the American tobaccos. But for non-American blends the smoke pH ranges from 6.5 to 7.8 when some of the nicotine molecules turn basic. Since at prior stage, the pH ranges around ~3.0 so, perhaps the act of de-protonation is willingly done to enhance the quality as well as to provoke addiction among the smokers. Since NH₃ is less powerful, so it is generally used instead of other strong alkalis to remove H⁺ from the nicotine. On the other hand, excessive NH₃ lowers the quality because of low burning nature. Thus an optimization is maintained during processing, keeping the pH ~ 5.5-6.5. But in many brands, the smoke pH is seen ~ 8.0 (Figure 4).

Nicotine is the molecule of major interest because it is the sole reason behind tobacco addiction. Owing to its immense significance, a brief knowledge about biosynthesis, absorption and physiologic role is provided for thorough coverage on the chemistry of tobacco smoking.

The chemistry of nicotine
Biosynthetic pathway: Nicotine is a plant alkaloid. Like many others, it is also bio-synthesized inside the plant to protect itself from the harmful effects of parasites and insects. Uniquely, the molecule possesses strong insecticidal and herbicidal properties and has been used for that purposes for sometime. It is the reason why several synthetic analogs are commercially manufactured and frequently used for the plant protection.

The biosynthesis of nicotine follows numerous enzymatic pathways that end by fusing the pyridine and pyrrolidone moiety (Figure 5). The pyrrolidone moiety is N-methyl cation derivative, prepared by the cyclization of oxidative deamination by diamine oxidase (DAO) on N- methyl putrescene (NMP). The NMP is formed from putrescene by catalytic action of N-methyl putrescene transferase (PMT). The pyridine moiety is possibly supplied from the NAD biosynthetic pathway. Although by using the labeled
isotope of nicotinic acid it is somewhat convincing that there could be an alternative possibility, that metabolic salvage pathway of NAD may contribute by donating the pyridine moiety. Figure 5 provides a brief illustration of nicotine biosynthesis. Nicotine comprises ~90% of the total alkaloids within tobacco leaves of which >99% exists as levorotary (S)-enantiomeric form and 0.1 – 0.6% as (R)-isoform. The (S)-enantiomer is physiologically more potent. Due to numerous undesired effects, the medicinal use of nicotine was discarded. But recently, some of the synthetic analogues have been found useful in arresting the progress of several neurological diseases. Besides the nicotine, all of its other family members Nornicotine, Anabasine, Anatabine and Myosmine, etc are also bio-synthesized following almost similar pathways.
Nicotine absorption in the body: The nicotine absorption in human depends on the ways tobbacos are used. In course of smoking, lung is solely the exposed organ to absorb nicotine and other substances from the aerosol. It absorbs quite efficiently, allowing them to pass readily into the blood stream. In less than 10 seconds nicotine reaches the brain. The absorption through lung is about the same as intravenous injection. Nicotine from the blood stream can cross the blood-brain barrier if it exists in the unionized form. It has a $pK_a \approx 8.0$, so the molecule is a weak base. In ionized or protonated form at $pH \geq 6.5$ its absorption would be low whereas at $pH \approx 7.5$ when most of the molecules are deprotonated, the pulmonary inclusion of nicotine becomes high (Figure 4). The high surface area of small alveoli spread throughout the lungs in conjunction with airways containing the bronchial fluid at $pH \approx 7.5$ enhances the nicotine dissolution. This helps permeate more deprotonated / uncharged molecules into the cells. Nicotine from smoke can also be absorbed through the saliva. In cigars, pipes or in European cigarettes, tobaccos exist at slightly higher $pH > 6.5$ which creates more unionized fractions enhancing the absorption of nicotine (Figure 4). Its level in circulation rises with puffing along with the time. The molecule reaches the brain within 7-10 seconds even after a single puff. The rapid rise in nicotine level in the brain insists smokers to be more dependent. Besides smoking, tobaccos used for the sake of chewing or snuffing are also conditioned at alkaline $pH$ in order to expedite the absorption through oral mucosa whose absorption rate is also high, but not greater than the lung alveoli. In those cases the plasma level reaches the plateau at ~ 30 min and starts to decline in ~ 2.0 hours. The rate of rise in brain is also found to be slower in comparison to the act of smoking.

Nicotine replacement therapy (NRT): To avoid the harmful effect of smoking this addictive agent, nicotine is alternatively delivered via chewing gums, lozenges or by transdermal patches; being termed as NRT. In those devices, the nicotine molecules are kept deprotonated / uncharged. Through intricate designing, the absorption rate is maintained low so that the build-up of level in circulation would be less than that achieved by the smoking. This slow build-up lowers the delivery rate and keeps the level low in the brain which helps reduce the abusing intentions by helping the smoker stay away from smoking. The absorption rate through oral mucosa from nicotine-gum or lozenges is seen higher than the transdermal patch. The patches are developed basing on the fact that uncharged nicotine molecule can permeate through the skin. They are built in a multilayered way and are impregnated with the nicotine in every layer. The delivery rate is controlled by altering the nature of polymer matrix or the number of layers being used. The different patches deliver at a different rate but in most cases there is a normal time lag of ~ 1.0 hour for nicotine molecule to appear in the circulation.

Besides the dermal patches, gums or lozenges, there are other medications available to combat the nicotine addiction. The common ones are Cytisine, Varencline and Bupropion. Cytisine acts as a partial agonist to nAcHR. Similar property is noticed for Varenicline whereas the action of Bupropion is different. Primarily it works as an antidepressant but at the same time it also helps in the cessation of smoking habit. Bupropion is a selective serotonin reuptake inhibitor (SSRI). It helps release dopamine and nor-epinephrine and inhibits their reuptake also. Further, the metabolite of Bupropion acts as nAcHR antagonist. Despite all those medicinal qualities, the drug has been withdrawn due to its side effect to induce migraine but it is occasionally used to treat ADD or ADHD among the children.

Chemistry of nicotine Metabolism: When it appears in the blood stream ($pH \approx 7.5$), ~ 31% of the nicotine molecules is unionized and ~ 69% stays.
in the ionized form. The binding of nicotine to plasma protein is > 5%. Smoking quickly delivers the nicotine within the pulmonary venous circulation. Thereafter, the flow passes into the left ventricle of heart and later enters inside the arterial circulation and finally permeates into the brain. The average delay time for nicotine molecule to reach into the brain is ~ 10 seconds. This rapid delivery rate produces intense pharmacologic reactions within the physiologic system\textsuperscript{36-39}.

Nicotine entering into the blood stream is metabolized mostly in the liver (Figure 7a and 7b). About 70-80\% within the circulation is converted to physiologically active component cotinine which is further metabolized afterward. The conversion to cotinine is catalyzed by the two enzymes (Figure 7a). The first one is CYP450-2A6 and the other is cytoplasmic aldehyde oxidase. But ~ 10-15\% of plasma nicotine appears in the urine, being unchanged. The other metabolite is nicotine N’-oxide (~ 7\%). About 3-5\% of the nicotine is converted to nicotine glucuronide. Approximately 4-7\% of it entering into the circulation follows another route to produce this N’- oxide derivative. The participating enzyme is flavin-containing mono-oxygenase 3 (FMO3) which produces diasteromers, 1’-1’-2’-(S)-cis and 1’-(S)-2’-(S)-trans configurations but that occurs only among the nonhuman primates. In humans, only trans configuration is identified in the urine\textsuperscript{35,38}. The nicotine N’-oxide is converted back to nicotine

![Figure 7(a) — Nicotine metabolism](image)

![Figure 7(b) — Cotinine metabolism](image)
inside the body, by the intestinal enzymes since it would not be metabolized any further. On the other hand, cotinine is extensively metabolized to different sets of compounds and finally excreted through urine (Figure 7b). It is already mentioned that among the major metabolites identified, about 10-15% of nicotine stays in the unchanged form. During metabolism, 35-40% is converted to trans-3'-hydroxycotinine, 12-18% undergoes glucuronidation and 5-7% switches to cotinine-N-oxide. The clearance rate of nicotine in humans is on the average, 1200 mL/min. About 70% of nicotine is metabolized in the liver and therefore, clears through it. The rests is cleared by the kidney by following almost similar pathways. The metabolic rate of cotinine is slower than nicotine and so is the clearance rate, ~ 45 mL/min. The clearance rate of some of its metabolites like, (3'R, 5'S)-trans-3'-hydroxycotinine is slightly higher ~ 82 mL/min but still much less than nicotine\(^9,46-49\). Cotinine is also pharmacologically active and recently used to treat depression, schizophrenia, and Alzheimer’s and Parkinson disorders\(^49,51\). It also binds, activates and desensitizes the neuronal nAChR\(^51\). Presumably, the few positive effects of nicotine can be attributed to its conversion to cotinine. Records show that cotinine treatment reduces anxiety and fear and unlike nicotine, it imposes no addiction. In that way, cotinine projects a positive safety profile\(^38,50\).

The pharmacokinetic studies at different ages demonstrate that nicotine clearance rate decreases at older age\(^39\) (>65 years) in comparison to the young adults\(^39\). The overall clearance level is lowered to ~ 23% whereas only the renal clearance, in particular drops to ~ 49%. The lesser blood flow at older age is sought to be the plausible reason since no change in CYP450-2A6 level is detected in the liver. The neonates also show reduced metabolism rate. The \(t_{1/2}\) is 3-4 times higher than the adults if exposed to the tobacco smoke whereas interestingly, the \(t_{1/2}\) for cotinine is same for the neonates, older children and adults. Many explanations regarding the blood flow performance are forwarded concerning the discrepancies of \(t_{1/2}\) for nicotine and cotinine at different ages. But one has to keep in mind that both drugs are primarily metabolized by the multiple enzymes other than the CYP450-2A6 alone. Neonates have slightly lower level of CYP450-2A6, CYP450-2D6 and CYP450-2E1 in their liver whereas the level of CYP450-2B6 is significantly much lower than adults or older children\(^39,48-51\).

Nicotine distributes itself inside most of the body tissues. The highest affinity is observed for liver, kidney, spleen and lung whereas the lowest is noticed in adipose tissues\(^39,40\). The molecule binds to brain / CNS tissues with high affinity. Interestingly, the binding capacity is higher in the case of smokers than nonsmokers\(^50,53\). This increment is hypothesized possibly due to the increase of nAChRs which express more owing to the repeated nicotine exposure\(^52,53\). In gastric tissues, nicotine predominantly accumulates into the gastric juice and saliva due to higher solubilization of protonated form\(^54\). It also accumulates in breast milk, amniotic fluid and fetal serum by being able to cross the placental barrier\(^55,57\). Its level in amniotic fluid and fetal serum is seen to be higher than in the maternal plasma. It is thus presumed that there is a possibility that nicotine may impose adverse physiologic effects on the newborns\(^56\).

In general, smoking delivers nicotine directly and quickly into the circulation. This rapid and repeated delivery helps build up high drug concentrations within the brain / CNS. The event asserts strong pharmacological actions and in turn allows in the developing of craving. Regarding the gender differences, studies indicate that nicotine and cotinine metabolism and their clearance are higher for the women than men. The use of oral contraceptive accelerates the clearance process\(^62\). For non-user women, the rate of nicotine and cotinine clearance is ~ 13% and 34% higher than the men whereas for the contraceptive users the rate increases to ~ 28% and 40%. The clearance rate is also seen higher during the pregnancy phase which is ~ 60% and ~ 140%. Pregnancy heavily increases the metabolism\(^55,57\). The rate is seen impaired in the case of kidney failure or liver problems\(^58,59\). In those situations, it drops below the 50% level. It is speculated that the accumulation of toxins lowers the enzyme CYP450-2A6 level by downregulating its expression\(^60,61\).

Besides diseases, various drugs also influence the nicotine metabolism. For example, Rifampicin (anti-tuberculosis), Dexamethasone (anti-inflammatory glucocorticoid), phenobarbital (anti-seizure), etc. expedite the nicotine metabolism by inducing CYP450-2A6 expression in the liver\(^62\). But at certain doses Rifampicin also inhibits the expression\(^57\). Other than the inducers, some drugs also act as inhibitors toward nicotine metabolism. The examples are Methoxsalen
(photo-chemotherapy of Psoriasis), Tranylcypromine (MAO inhibitor), Tryptamine (neuro-modulator) and Coumarin (anti-coagulant). β-Nicotyrine, another tobacco alkaloid belonging to the nicotine family also efficiently inhibits the CYP450-2A6. For that reason it is often used to combat the smoking habit.

Physico-chemical interaction between nicotine and nAChR: Nicotine is a powerful stimulant and also a nAChR agonist within parasympathetic neurons. In human, nAChRs are spread all over the body, more intense within the nervous systems like brain, spinal cord or other nerve tissues. The receptor, nAChR belongs to the family of ligand gated ion channel (LGIC) existing on both sides of the pre and postsynaptic membranes of neuromuscular joints. There are several isoforms of nAChR built from the pool of 9α (α2 → α10) and 3β (β2 → β4) subunits which could be either homo or hetero pentameric by nature (Figure 8). The nicotine binding sites are

![Diagram of nAChR isoforms](image-url)
located at the subunit surfaces. The binding affinity is high \( K_D \sim 1.0 \times 10^9 \text{ M/L} \) for heteromeric like \((\alpha_4\beta_2)\) but less \( K_D \sim 4.0 \times 10^6 \text{ M/L} \) for the homomeric \((\alpha_7)\) form (Figure 8). Even with lesser affinity toward homomeric form, the interaction creates numerous neurological effects. Thermodynamically, the binding interaction is seen to be enthalpy \((\Delta H)\) driven, which points to the alteration of receptor’s conformation after docking of the ligand at binding sites. The event helps opening of the ion channels and allowing the inflow and outflow of cations.\(^{54,56}\) Evidence indicates that binding primarily occurs due to the formation of H-bond and also involving close packing of the hydrophobic aromatic groups. It is shown that the +vely charged nitrogen atom of pyridine in \((S)\)-nicotine enables to form the H-bond more efficiently. In that regard, the \((S)\) – conformer is more favorable (10-100% potent) compared to the \((R)\)-enantiomer. The receptor works interconvertibly, switching to the different conformational states. Usually, agonist binding stabilizes the open desensitized state allowing the +vely charged ions to flow across the membrane by letting the Na\(^+\) to enter and K\(^+\) out, while maintaining the net flow of +ve ions inward.\(^{64}\) Characteristically, nAChR is a non-selective cation channel so it allows the flow of different +vely charged ions including Ca\(^{2+}\), but that property depends on the subunit combinations. The membrane conductance during ion flow varies from 50-110 Po\(^{1}\) which also depends on the subunit configurations. The channel opening time is less than ~ 1.0 milli-second until the agonist diffuses out from the bound phase. Recently, much interest has been focused on homomeric \(\alpha_7\) and heteromeric \(\alpha_4\beta_2\) subtypes in studying the nicotine addiction, ADHD, schizophrenia, Parkinson's, cognitive impairment and Alzheimer's disease.\(^{65,67}\) The compound, ABT-418 synthesized by theAbbott Corpn. (Illinois, US) is nAChR agonist displaying high affinity toward both \(\alpha_4\beta_2\) and \(\alpha_7\). It is useful for treating ADHD and Alzheimer's disease although it cross-reacts with the \(\alpha_5\beta_2\) isoform of nAChR and 5HT\(_3\) (Serotonin) receptor but ABT-089 is seen more selective toward the \(\alpha_5\beta_2\) but much less toward \(\alpha_4\beta_2\) and not at all toward \(\alpha_7\) or \(\alpha_3\beta_4\), almost like the behavior of original nicotine molecule (Figure 9).\(^{67,69}\) Structurally both mimic the nicotine molecule having quaternary nitrogen within the pyrrolidone moiety which is distinctively essential for molecular interaction with the receptor in order to exert any physiologic effects (Figure 9). So the research on nicotine-nAChR interaction becomes an important issue not just to prevent the smoking habit but also to control the progression of many neurological diseases. Numerous subtypes of functional nAChRs are known to exist within the mammalian brain neurons. For example, inside the Hippocampus, \(\alpha_7\), \(\alpha_4\beta_2\) and \(\alpha_3\beta_4\) are mainly expressed. Hippocampus is involved in consolidating the information from short term to long term memory and also helps in the course of spatial navigation. The presynaptic region expresses \(\alpha_7\) and \(\alpha_4\beta_2\) which sits on the metabotropic (mGluR or GABA-ergic) receptor containing nerve terminals. Nicotine at low doses activates the mGluR of presynaptic nAChR within the hippocampus releasing both excitatory and inhibitory neurotransmitters.\(^{10}\) The phenomenon signifies a possible link in the case of addictive smokers. The post synaptic nAChRs express subunits like \(\alpha_4\), \(\alpha_5\), and \(\beta_2\) within the hippocampal neurons.\(^{71}\) They facilitate fast excitatory synaptic transmission. It is also thought that the action of pre and post-synaptic nAChRs may cause any long term changes needed for the learning behavior and also for the functioning memory. The long-term potentiation (LTP) is due to the enhancement of long-lasting and efficient increase of the memory functions. It is likely that nicotine might play a significant role in the LTP of memory by activating the nAChR. The systemic application of nicotine not only induce the LTP, it also enhances the effect in presence of other nAChR agonist as observed during several animal experiments. Presumably, the homomeric \(\alpha_7\) within dentate gyrus inside the hippocampal region and nicotine are involved in this LTP induction process. In a way nicotine and its agonists play a positive role in the memory functions.\(^{71}\)

![Figure 9 — Drugs synthesized for treating Alzheimers’ and Parkinson disorders. ABT-418 → nAChR agonist having high affinity toward \(\alpha_4\beta_2\) and \(\alpha_7\), but heavily cross-reacts with \(\alpha_5\beta_2\) isoform and 5HT\(_3\)(Serotonin) receptor. ABT-089 → moreselective toward \(\alpha_4\beta_2\) but much less toward \(\alpha_5\beta_2\) and not at all for \(\alpha_7\) or \(\alpha_3\beta_4\).](image-url)
**Physiological role of nicotine:** Nicotine offers multiple roles in physiology which could be broadly classified into two classes. One is the reinforcement, displaying tolerance, physical dependences while the other is pharmacological, modulating the mood, food intake or appetite and task performance. Essentially, it helps continue the compulsive habit of smoking, rather say, addiction caused by the activation of nAChRs while at the same time modulating the dopaminergic neurons inside VTA of the brain in order to be rewarded\textsuperscript{71}. It has been established that nicotine helps induce LTP by interacting with the dopamine neurons inside VTA through the enhancement of glutamate release while activating the $\alpha_7$ isofrom of nAChRs. This enactment is the exact fact behind the LTP induction by nicotine\textsuperscript{71}.

Considering the peripheral actions, nicotine increases blood pressure and heart rate through myocardial contractility by catecholamine released from the adrenal medulla. The depolarization of sympathetic nerve endings during nicotine-nAChR interaction stimulates the Ca\textsuperscript{2+} influxes via voltage dependent N-type Ca\textsuperscript{2+} channels which in turn triggers the catecholamine release through exocytosis. Later, that directly acts on the heart inducing tachyphylaxies through the release of norepinephrine from sympathetic neurons\textsuperscript{72,73}.

It is noticed that nicotine is also involved in the development of gastro-intestinal diseases (peptic ulcers, delaying the cure of gastric wounds) and to a certain extent carcinogenesis also\textsuperscript{74,75}. Regarding the carcinogenesis, it shows some signs of mitogenic and angiogenic effects on the vascular tissues by inducing atherogenic genes in coronary artery endothelial cells. It is identified that long term smoking causes vascular injury owing to its continuing interaction with the nAChR creating endothelial dysfunction and subsequent pathogenesis for the atherosclerosis\textsuperscript{75,76}. Although nicotine itself is not assigned to be a carcinogen by the FDA, but several recent studies indicate that it plays an indirect role by obstructing the apoptosis while enhancing tumor growth through the activation of several mitogenic and growth factors like 5-LOX and EGF. Reports on animal studies further indicate that nicotine can enhance the growth and metastasis of certain tumors\textsuperscript{77}. The in vivo generation of N′-nitrosonornicotine, a recognized carcinogen is suspected to be an underlying cause\textsuperscript{78}.

Regarding ulceration, epidemiological studies indicate that nicotine from smoking enhances the incidence of peptic ulcers and its relapse rate\textsuperscript{79-81}. But the effect of nicotine on gastric acid secretion is controversial. Several workers claim that nicotine stimulates the basal acid secretion via the activation of muscarinic acetyl choline receptor which may activate the parietal cells in stomach. But it is also noticed that nicotine potentiates histamine induced acid secretion whereas inhibits the pentagastrin stimulated acid production. Interestingly, it also increases pepsinogen secretion from the chief cells\textsuperscript{81}. Further, nicotine shows the delaying effect in healing the gastric wounds by suppressing the mucosal restoration while inhibiting the enzyme, ornithine decarboxylase needed for intestinal tissue repair\textsuperscript{82,83}. But aside those adversarial effects, it is interesting to note that nicotine shows preventive role on the progression of UC\textsuperscript{83}. Recent clinical trial supports the fact very strongly.

A long list of cancers are seen to be connected to the nicotine use. The list includes cancers of lung (small-cell and non-small-cell carcinomas), head and neck, pancreatic, gall bladder, liver, colon, breast, cervical, urinary bladder and kidney\textsuperscript{84-86}. The stimulation of various nAChRs might activate several different cell signaling pathways which may cause tumorigenic effects. The mutagenic role of nicotine is suspected for its ability to damage the genome which may facilitate the tumorigenic growth. Further, genetic polymorphism involved in nAChR encoding might also influence the individual’s susceptibility towards any harmful action of nicotine\textsuperscript{87}.

**Chemical effects of other tobacco ingredients**

Besides nicotine, there are other ingredients in the tobacco smoke including those from the additives that are also recognized as being harmful to the health. A few have been previously mentioned. Among them, benzo[a]pyrene, 4-ABP, NA, formaldehyde and EO are characterized as the most powerful carcinogens. Each one can induce varieties of cancers (Table II)\textsuperscript{87}. Benzo[a]pyrene might not have the direct harmful effects. But its oxidized metabolite benzo[a]pyrene-7,8-dihydrol-9,10-epoxide actually imposes the toxic and mutagenic effects by using this active intermediate (Figure 10). It induces urinary bladder and pulmonary adenomas by interacting with various macromolecules also intercalating within the DNA\textsuperscript{88}. Like the benzo[a]pyrene, 4-ABP and NA are also enabled to form adducts with the nucleotides (guanosine or adenosine) inflicting damage to the DNA and inducing various cancers (Figure 11, 12 and 13). Both ABP and NA are seen to be involved in creating the hepatic and bladder cancers. Between the NA-1 and 2, the second isomer is suspected to be more harmful regarding their
Figure 10 — Oxidation of Benzo[a]pyrene

Figure 11 — Reaction products of 4-ABP with the DNA nucleotides [A = d-ribose group linked to the DNA strand]
Figure 12 — Reaction of 1-Naphthylamine with Guanosine [A = ribose linked to DNA]

Figure 13 — Reaction of 2-Naphthylamine with Guanosine and Adenosine [A = ribose linked to DNA]
The DNA adduct created by 1-NA is not detected in the urinary bladder but identified only inside the hepatic DNA. Normally, two adducts are formed by the - NA, e.g., N-(deoxyguanosine-O\(^6\)-yl)-1-NA and 2-(deoxyguanosine-O\(^6\)-yl)-NA. The former is seen 20 times more potent in creating bladder tumor. In case of 2-NA, there is a significant amount (~50%) of ring-opened product (ring-opened N-(deoxyguanosine-8-yl)-2-NA) is formed while reacting with guanosine\(^90\). But in addition, there are three other products produced during reaction with guanosine and adenine, e.g., N-(deoxyguanosine-8-yl)-2 NA, 1-(deoxyguanosine-N\(^2\)-yl)-2-NA, 1-(deoxyguanosine-N\(^6\)-yl)-2-NA (Figure 11, 12 and 13).

As for formaldehyde, it is a well-established cancer causing agent. It is listed as a human carcinogen according to the Second Annual Report on Carcinogens in 1981 provided by the National Toxicology Program of Health and Human Services of US Government, presented with sufficient evidence based on the laboratory studies on different animals. In the 12\(^{th}\) report based on human studies it is declared as a potential carcinogen for human\(^90\). It creates nasopharyngeal, sinonasal and lymphohematopoietic cancers especially the myeloid leukemia to those who are continually exposed to it for a long period of time. Besides being known to be a carcinogen it is known as a strong teratogen. Chemically, the >C=O moiety of the molecule is reactive towards any primary -NH\(_2\) or -SH group causing irreversible damage to the native proteins, enzymes and nucleic acids. It is the prime reason behind carcinogenesis. The incidence of nasopharyngeal cancer is seen to be much higher than the other types.

The cancer causing behaviour of benzene is complex because it follows complicated metabolic pathways. Benzene causes acute myeloid leukemia but frequent instances of non-Hodgkin lymphoma, myelo-dysplastic syndrome and other haemopoietic diseases are also observed\(^91\). The epidemiological studies indicate that even at the EPA permissible level (~1 ppm), it induces hematotoxicity if exposed regularly for a long period of time. It affects several genes capable of inducing abnormalities within hematopoietic stem cells, altering the nature of proliferation and differentiation\(^92\). The effects are particularly mediated via oxidative stress with the production of reactive singlet oxygen species lowering the immune-surveillance and thereby producing the leukemic stem cells. Its metabolism proceeds primarily within the liver and lung. The oxidation reactions involve cytochrome enzymes (CYP450) leading to the production of benzene oxide which afterward rearranges to phenol and later to benzoquinones, 1,2- and 1,4- isomers along with the production of hydroquinone and other oxidative products (Figure 14). Further, the tautomer of benzene oxide, oxepin is seen to undergo ring opening by CYP450 enzymes producing reactive \(E,E\)-muconaldehyde (CHO–CH=CH–CH=CH–CHO) (Figure 14). The quinones and muconaldehydes are strong electrophiles which readily react with the proteins, peptides and nucleotides disturbing their normal cellular functions and subsequently inducing carcinogenicity\(^93\). The harmful diol-epoxides are also classified as being carcinogens\(^94\). It is established that benzene and its metabolites can create chromosomal abnormalities within peripheral blood lymphocytes in humans if exposed chronically which could induce acute myeloid-lymphoma\(^90,93\).

Records indicate that EO, a potent alkylating agent is also involved in carcinogenesis\(^95,96\). Exposure to EO quickly alkylates the macromolecules like DNA, proteins and others\(^90\). Therefore, its presence within the smoke causes harmful effects among the smokers. When studied in animals, significant levels of death could be observed due to the occurrence of mononuclear cell leukemia, peritoneal mesothelioma as well as brain glioma. Additionally, the incidences of epithelial hyperplasia and adrenal pheochromocytomas are also occasionally observed owing to the long exposure. The oxygen in EO readily takes part in the alkylation event in presence of strong nucleophiles like –NH\(_2\), =NH, –OH, –SH, etc.

![Ethylene oxide](image)
**Table IV — List of health problems created by cigarette smoking**

<table>
<thead>
<tr>
<th>Number of Health problems</th>
<th>Diseases / Disorders</th>
<th>Number of Health problems</th>
<th>Diseases / Disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mood stimulation</td>
<td>2</td>
<td>Poor vision</td>
</tr>
<tr>
<td>3</td>
<td>Appetite loss</td>
<td>4</td>
<td>Anxiety &amp; irritability</td>
</tr>
<tr>
<td>5</td>
<td>Coughing</td>
<td>6</td>
<td>Increases cold and flu attack</td>
</tr>
<tr>
<td>7</td>
<td>Terribly affects COPD</td>
<td>8</td>
<td>Increases rate of lung cancer</td>
</tr>
<tr>
<td>9</td>
<td>Induces bronchitis</td>
<td>10</td>
<td>Constricts blood vessels causing high blood pressure</td>
</tr>
<tr>
<td>11</td>
<td>Enhances blood clotting</td>
<td>12</td>
<td>Increased blood cholesterol</td>
</tr>
<tr>
<td>13</td>
<td>Increases chances of blood cancer</td>
<td>14</td>
<td>Causes cardiac problems</td>
</tr>
<tr>
<td>15</td>
<td>Causes yellow finger</td>
<td>16</td>
<td>Badly stains teeth</td>
</tr>
<tr>
<td>17</td>
<td>Produces wrinkle in the skin</td>
<td>18</td>
<td>Creates diabetes complications</td>
</tr>
<tr>
<td>19</td>
<td>Creates erectile dysfunction</td>
<td>20</td>
<td>Induces infertility in both men and women</td>
</tr>
<tr>
<td>21</td>
<td>Induces early menopause</td>
<td>22</td>
<td>Enhances chances of cervical cancer</td>
</tr>
<tr>
<td>23</td>
<td>Creates problems in pregnancy</td>
<td>24</td>
<td>Creates severe problems in the newborns</td>
</tr>
</tbody>
</table>

**Major health problems faced from tobacco smoking:** Table IV lists the major health problems that are often faced due to regular smoking of cigarettes. They are classified into two categories: instantaneous and long term. The instantaneous ones are coughing, anxiety, mood stimulation and increase of blood pressure whereas the latter category, perhaps the most virulent ones are cancers, bronchitis, cardiac problems, COPD, birth-defects, etc. Besides smoking, the chewing of
tobacco also incorporates a number of health problems, especially inducing throat or esophagus cancers.

**Biomarker**

The extent of exposure either from the environment or due to direct smoking of cigarettes is measured by analyzing the physiological fluids like blood, urine or saliva. The widely used markers are either cotinine or 3-ethyl pyridine. The 3-ethyl pyridine is more volatile than nicotine and produced during its pyrolysis. It is mainly used for those who are exposed to indirect tobacco smoke whereas cotinine is measured in the saliva, blood or urine of regular smokers. The t\(_{1/2}\) of cotinine in plasma is ~ 16 hours. It is a stable metabolite and its levels in saliva, blood and urine correspond with each other very well. For a regular smoker, plasma nicotine level rises in the morning but levels off later during the day. Only ~ 15% change in the level is observed at night. As mentioned before, ~ 80% of the nicotine molecules are converted to cotinine. That is why it is used as a potential biomarker which is further metabolized to 3’-hydroxylcotinine. All are conjugated later to glucuronides leaving a tiny bit as being unchanged (Figure 7b). Besides plasma, the urinary excretion of major nicotine metabolites can be measured and summed up for estimating the average nicotine consumption\(^{39,96}\).

**Summary**

The habit of smoking is universally considered to be harmful for health, so is the chewing of tobacco leaves. Obviously, smoking is the worst of all, since it introduces innumerable number of hazardous ingredients which are harmful and carcinogenic as well. The main component is nicotine, an alkaloid which provokes addiction by penetrating into brain, crossing the blood-brain barrier. The mechanism behind nicotine addiction is revealed. Besides imposing addiction, nicotine also produces other serious effects. The other very harmful components that get inside through inhalation is the tar-products from the pyrolysis of tobaccos. It has been identified that ~ 7000 items produced by pyrolysis are extremely health threatening. In order to avoid them, alternatively the NRT by chewing tobacco or using nicotine gums or dermal patches are practiced. Admittedly, it offers some protection but not totally since nicotine also has harmful effects. The article here describes chemical and physiological effects of nicotine and other chemicals. But despite the harmful effects of nicotine, some of its analogs offer a few positive clues towards controlling several neurological diseases like Parkinson’s, Alzheimer’s and even ADD. So, while avoiding the direct use of nicotine, some of its synthetic mimics have been launched for medicinal purposes.

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**References**


