Original Article
ISSN (Print) : 0971-6580
Toxicology International, *Vol 28(4), 279-309, October -December 2021, DOI: 10.18311/ti/2021/v28i4/26489* *ISSN (Online) : 0976-5131*

Chemical Components in Smokeless Tobacco Products and Impact on Health

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History: Received 2020-12-03; Accepted 2021-06-11; Published 2021-12-22

Abstract

Smokeless Tobacco [SLT], a non-combustible form of tobacco, is consumed by 350 million people in 133 countries across the globe. Worldwide, Smokeless Tobacco products vary greatly in their formulations and chemical composition. Understanding of toxic and carcinogenic constituent variations in such products can provide valuable insights for the development of effective tobacco control policies. Though the assessment of SLT products has been done earlier, the information is not available in an inclusive and handy format as entire profiling. Hence, there is a vital need to develop a one-stop information source providing comprehensive information on SLT products. PubMed and Google scholar databases were systematically searched from 1995 till April 2020 for observational studies on Smokeless Tobacco products and their chemical components. The included studies were evaluated and data were extracted and reviewed.A wide variation was noted in the association of various diseases and specific Smokeless Tobacco product constituents based on their nature and inherent toxicity. The majority of chewing tobacco products displayed a higher risk for users.This review emphasizes the significant positive association of Smokeless Tobacco product components with health hazards. Pathways estimates for smokeless tobacco-associated disease need further analysis. The profiling of Smokeless Tobacco products also requires multi-centric well-designed studies. Further, the information would be a guide for researchers interested in the components of SLT products.

Keywords: Biological Mechanism, Disease Pathway, Hazard, Profiling, Smokeless Tobacco, Tobacco Constituents

1. Introduction

Unlike smoked tobacco, Smokeless Tobacco products [SLT] are used orally, which results in the absorption of harmful chemicals through the mucous membrane[1]. The whole world uses SLT products but in different forms that are differentiated mostly in appearance, chemical composition and toxic emission[2,3]. In nature, about 70 species of tobacco are present but among them, only a few are used regularly for smoked or Smokeless Tobacco products. By chemical analysis of tobacco alkaloids and the level of nicotine, we can find different tobacco species present in products[4]. In the overall world, *Nicotiana tabacum [N. tabacum]* species of the tobacco plant are mostly used in commercial tobacco products but in Asia, South America and Africa, *Nicotiana rustica [N. rustica]* is more often used[5]. In India, *N. tabacum* is used for the preparation of smoking tobacco but Smokeless Tobacco has *N. rustica* having an increased level of alkaloid and nicotine than *N. tabacum*[6]. Moreover, both *N. tabacum* and *N. rustica* are present in some tobacco products such as "khaini". A high concentration of alkaloid anabasine is present in tobacco species *N. glauca* which is used in SLT toombak. Glauca species is known to be highly carcinogenic.

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Approximately 4000 chemical compounds of different classes that are highly carcinogenic are present in SLT products[7]. Among all carcinogens, tobacco-specific nitrosamines are potent because of their concentration and involvement in the induction of oral cancer. Other carcinogens present in SLT include PAHs [Polynuclear Aromatic Hydrocarbons], toxic metals, volatile aldehyde and inorganic compounds. A variety of toxic metals that either enter during tobacco processing are absorbed by the tobacco plants from the soil. Depending upon their carcinogenicity, they are classified as Group 1, Group 2B and Group 3. The concentration of toxic metals varies among different brands of SLT and this toxicity has a direct correlation with the disease burden of the individual body.

Before use in products, tobacco undergoes different processing techniques. During curing and other tobacco processes, the chemical composition of tobacco undergoes substantial changes. In curing, the percentage of starch present in tobacco is decreased excessively but the amount of reducing sugars is increased by 100%. Nicotine and protein concentration is decreased. Before the fermentation process tobacco leaves contain approximately 50% carbohydrates and protein. Tobacco leaves fermentation causes carbohydrates to diminish. Another component that are present include 0.5-5% alkaloids, which contain 85 - 95% of nicotine, polyphenols [0.5-4.5%], carboxylic acid [0.1-0.7%], terpenes [0.1-3%], ketones, aldehydes, amines and at least 30 other metallic compounds. Because of decreased content of polyphenols and carbohydrates during the fermentation process, additives are added to improve taste and flavor and to fulfill the consumer's requirement[8] that include licorice, molasses, ethyl or methyl salicylate, benzyl benzoate, menthol and coumarin[9]. As an antimicrobial agent in tobacco, ascorbic acid is added whereas sodium propionate serves as a fungicide. Moreover, to control nicotine delivery, pH is raised using additives such as sodium carbonate, ammonium carbonate and ammonia[10]. The addition or formation of different chemicals in tobacco processing are found to be associated with a large number of health effects and diseases.

2. Aims and Objective

Significant numbers of studies and many literature reviews have been published, mostly confined to a single health effect or an individual component. An appropriate approach for the entire profiling of the component effect does not appear in any reviews. So, the study was carried out with an aim to compile the entire profile of chemical components in Smokeless Tobacco products and their associated biological mechanism. The objective of the review is to provide narrative and tabular summaries of the results of studies for profiling of components properties and effects. It includes biological pathways leading to various diseases and the hazardous effects of the particular constituents. Also, an attempt was made to quantify the major health effects associated with SLT use.

3. Methodology

PubMed and Google Scholar databases were systematically searched from 1995 till April 2020 for observational studies on Smokeless Tobacco products and its chemical components. Authors undertook/performed a literature search in the electronic databases using the following strategy: [Chemical components or tobacco constituents or SLT ingredients] and [Nicotine or TSNA or Ammonia or Coumarin or Tobacco Additives or Metals or PAH] and [Smokeless Tobacco Products]; with regard to effect and disease pathway of chemical components in SLT. Additionally, we look over reference lists of related articles and searched our archive for further potentially relevant publications, not identified through the electronic search. Subsequently, the included studies were evaluated and data were extracted and reviewed.

4. Results

The constituents of SLT are represented in Figure 1, which are further described in the following subsection

4.1 Additives and Flavourings

4.1.1 Aflatoxin B1 [AFB1]

Exposure to aflatoxins in cigarettes may be mitigated by

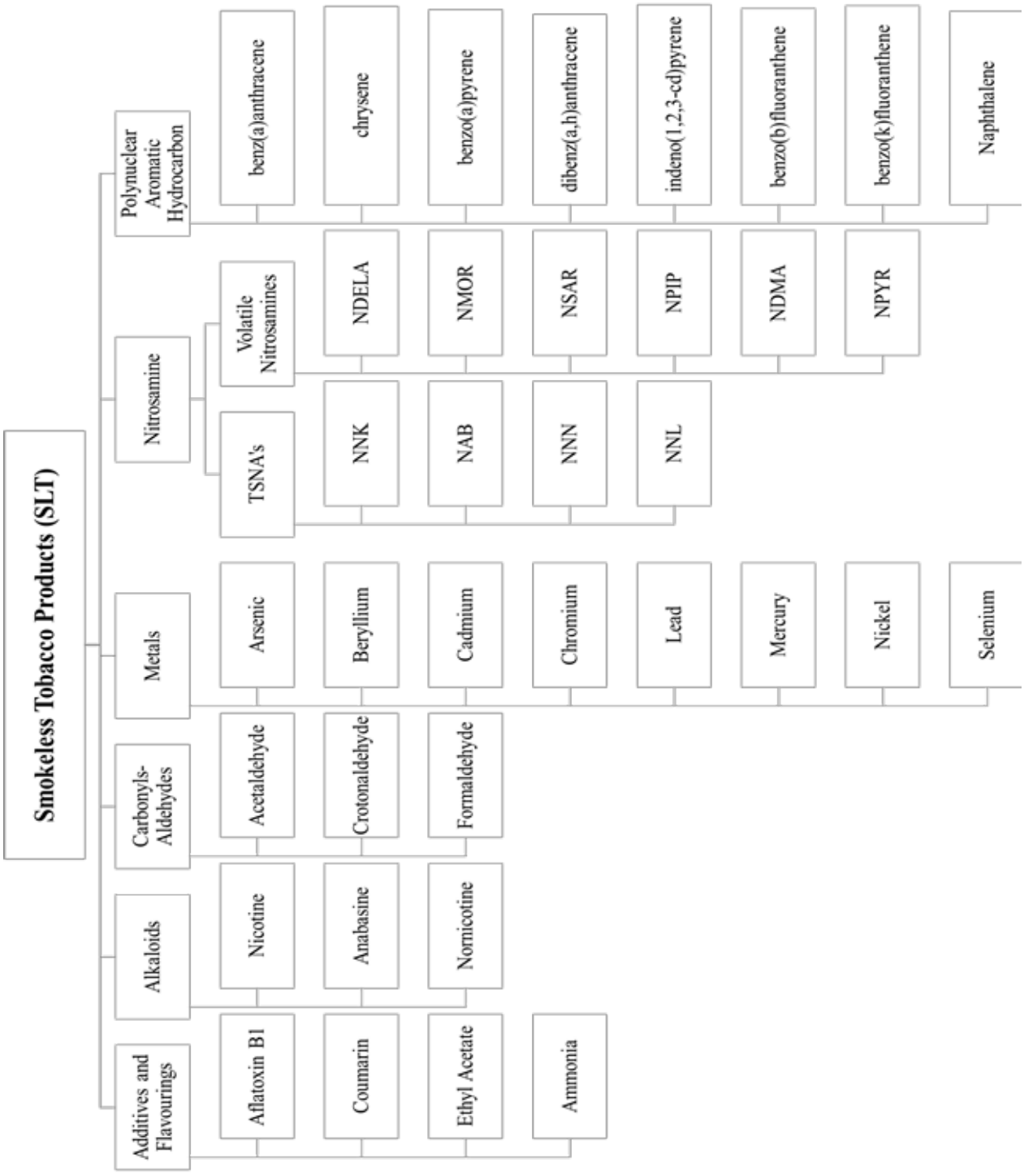


Figure 1. SLT chemical Constituents.

Table 1. Description and effects of Aflatoxin B1 [AFB1]

Data		References
Description		
Property	Aflatoxins are toxic growth products [mycotoxin] A known contaminant on flue-cured tobacco leaves Found in environmental tobacco smoke [ETS]	[12]
		[13]
IARC Classification	Group 2B	[14]
Chemical Classification	As dibenzo-furofurans highly oxygenated heterocyclic compounds	[15]
Nature	Carcinogen [CA] teratogen, mutagen, immunosuppressant, and potent inhibitor of protein synthesis	[11, 13, 16]
Invading Fungus	Aspergillus flavus	[11]
Favored Environment	Hot and humid climate	[13]
Level of Aflatoxin		
Daily exposure	1 ng/Kg	[17]
Maximum Residue Level	Commodities [20 ppb]; Milk [0.5 ppb]	[13, 18]
In vivo animal models		
Mice Model	pulmonary carcinogen [Lung tumor]	[12,13]
Effects		
Toxicity	mutations in the p53 tumor-suppressor gene as well as "ras" mutations, which are involved in the majority of human cancers	[13]
Target organ	Liver [via dietary exposure]	[13]
Symptoms	Weight loss, Impaired immune system	[11]

their potential destruction in the combustion process; however, aflatoxins present in a smokeless product are likely to be introduced into the body via ingestion[11]. The description of aflatoxin is summarized in Table 1 and mechanism is illustrated in Figure 2.

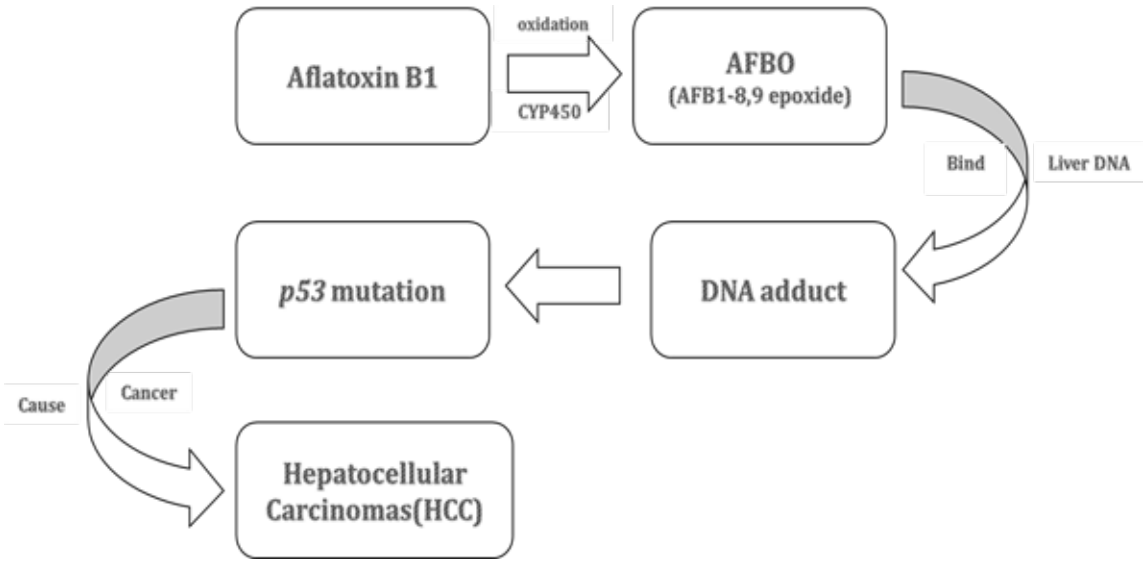


Figure 2. Illustrates Mechanism of Aflatoxin Toxicity.
Cytochrome -P450 enzyme metabolized AFB1 to the reactive intermediate AFB1-8, 9 epoxides [AFBO]. Binding of AFBO to liver cell DNA, results in DNA adducts, which interact with the guanine bases of liver cell DNA and cause a mutational effect in the p53 tumor suppressor gene which may lead to HCC.

4.1.2 Ammonia

Introduction of ammonia-containing compounds during tobacco manufacturing may affect the organoleptic and mechanical properties of the tobacco. The description of Ammonia is summarized in Table 2.

Table 2. Description and effects of ammonia

Data		References
Description		
Use	Chemical additive found in tobacco filler Listed as non-exhaustive priority list of 39 tobacco contents	[19]
Nature	Respiratory Toxicant [RT]	[16]
Properties	corrosive and exothermic	[19]
Level of ammonia		
Blood- Healthy Adult	15 to 45 micrograms/Dl	[20]
As taste enhancer	0.25% concentration	[21]
Tobacco [per gram]	0.87 to 2.41 mg	[22]
High level effects		

Adult	Behavioral/ Psychological problems- confusion, tiredness, and possibly coma or death Injury to the mucosa of the eyes, skin, oral cavity, and respiratory tract	[19, 23]
Child	Seizures, breathing trouble, lower response, and potentially death	
Mechanism of action	Ammonia interacts immediately upon contact with available moisture in the skin, eyes, oral cavity, respiratory tract, and particularly mucous surfaces to form the very caustic ammonium hydroxide. Ammonium hydroxide causes the necrosis of tissues through disruption of cell membrane lipids [saponification] leading to cellular destruction. As cell proteins break down, water is extracted, resulting in an inflammatory response that causes further damage.	[24]
Other Effects		
pH, Nicotine release and absorption	Increases product alkalinity, which in turn increases the amount of unprotonated nicotine Unprotonated["free-base"] nicotine is lipophilic and is absorbed more quickly into the bloodstream than its protonated form Thus, for Smokeless Tobacco it seems that an increased pH enhances nicotine absorption in the mouth Also, it increases nicotine dependence in cigarette smokers	[25, 26]

4.1.4 Coumarin

Despite known severe toxic and carcinogenic risks to humans, coumarin has long been used by the tobacco industry as an additive.The description of coumarin is

Table 3. Description and effects of Coumarin

Data		References
Description		
Property	flavourings agent in SLT	[27]
IARC Classification	Group 3	[14]
Nature	Banned in food	[16]
Class	Semi-volatile Lactone	[28]

Table 3 Continued

Level of Coumarin		
Daily Exposure at	0.06 mg/Kg/ day [dietary and cosmetics]	[29]
Safe intake level	0.1 mg/kg body weight [b.w.] per day	[30]
Tobacco naturally present	<1 ppm	[28]
fire-cured tobacco products	17,400ppm	
Effects		
Exposure via	Essential oils such as Cinnamon leaf [40,600 ppm] Bark oil [7000ppm]	[28, 29]
Symptoms	Coma and death [including liver deterioration and blood vessel dilation]	
Toxic effects	On the nervous system, heart, blood vessels, and liver of animals as well as inducing cancerous tumors and toxic conditions in humans.	
	Enters into human circulatory systems unhindered by the body's normal detoxification processes and are often more toxic than the original substance.	

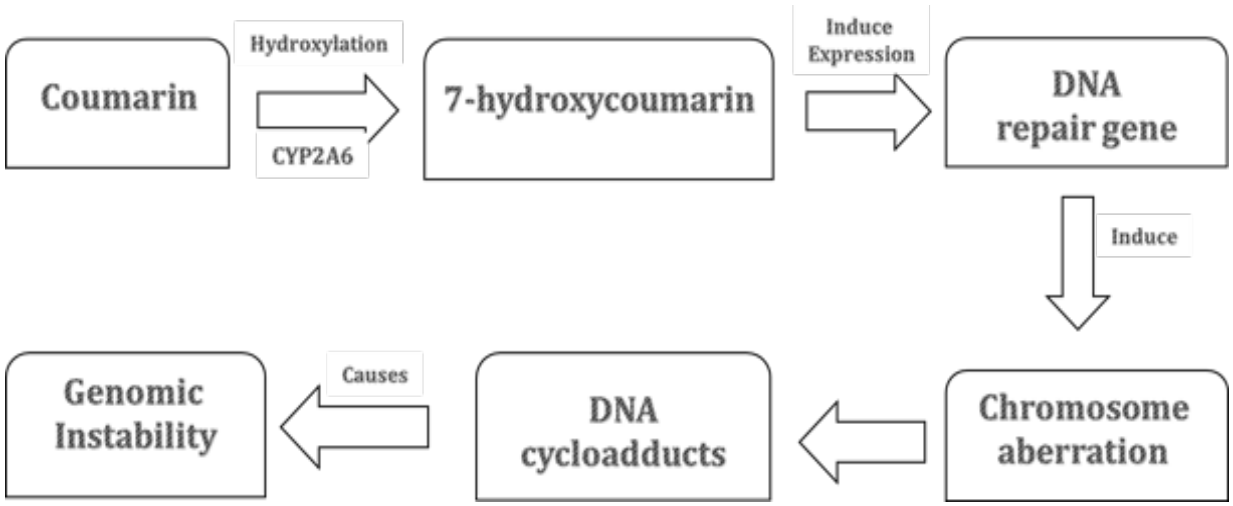


Figure 3. Illustrates Mechanism of Coumarin Toxicity.
Metabolism of coumarin in human and animal is qualitatively similar. Coumarin is metabolized by the enzyme CYP2A6 [enzyme: Cytochrome P450 2A6] to form 7-hydroxycoumarin [7-HC]. This induced expression of the DNA repair gene which is responsible for induction of chromosome aberration [CA] in cells, formed DNA cycloadducts which ultimately causes mutations and genomic instability.

summarized in Table 3 and mechanism is illustrated in Figure 3.

4.1.4 Ethyl Carbamate [EC]

EC or urethane, is the ethyl ester of carbamic acid. The description of ethyl carbamate is summarized in Table 4

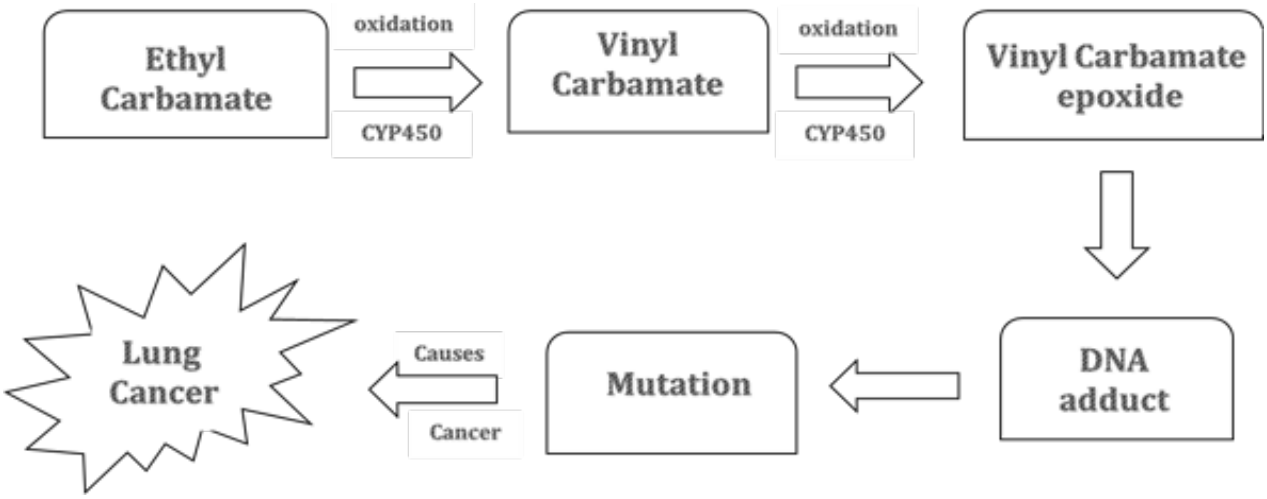


Figure 4. Illustrates Mechanism of Ethyl Carbamate Toxicity.
Ethyl carbamate metabolism is a two-step oxidation process, EC undergoes oxidation to form VC [vinyl carbamate], with a second oxidative step that results in the subsequent conversion of VC to VCO [Vinyl Carbamate epoxide]. Both oxidative steps are mediated by CYP2E1 [Membrane protein: Cytochrome P450 2E1] which results in the formation of an epoxide, and the subsequent production of DNA adducts and associated mutations that ultimately cause lung cancer.

Table 4. Description of Ethyl darbamate present in Smokeless Tobacco

Data		References
Description		
Property	Naturally occurring chemical and a by-product on fermentation	[31]
IARC Classification	Group 2A	[14]
Nature	Carcinogen [CA], Reproductive or Developmental Toxicant [RDT]	[16]
Class	Listed as a Hazardous Air Pollutant [HAP]	[32]
Level of Ethyl Carbamate		
Tobacco	374.1 ng/g	[33]

Table 4 Continued

Alcoholic Beverages	Wine: 30 µg/L [Canada/ Czech Republic]& 15 µg/L [USA] Fruit Brandies : 400 µg/L [Canada/ Czech Republic]	[34]
Effects		
Toxicity	Low mutagenicity in bacterial cells and gives positive responses in some mammalian cell assays for chromosomal aberrations, sister chromatid exchange and micronucleus induction	[35]
Rodents	Induce tumors in various organs, probably via the formation of the metabolite vinyl carbamate	[36]
Target Organ	Liver and Lung	[35]
Plants	Chromosome Translocation	

and mechanism is illustrated in Figure 4.

4.2 Alkaloids

Tobacco contains a number of structurally related pyridine alkaloids. The description of alkaloids is summarized in Table 5.

Table 5. Description and effects of alkaloids

Data		References
Description		
Property	Organoleptic [Stimulant]	[37]
Fraction of total alkaloids	Major: Nicotine ~95% Minor: Nornicotine, anabasine, anatabine~4% to 5% of total alkaloids Other alkaloids are demethylated derivative of nicotine	[38]
Nature	Anabasine, Nornicotine: AD [Addictive]; Nicotine: Reproductive and developmental Toxicant [RDT], AD	[16]
Forms of Nicotine	Exist as a free-base [uncharged] or as protonated salts Primarily present in its protonated form [at low pH] is non-volatile and pass poorly through membranes thus, less easily absorbed into the lungs Free-base nicotine is semi-volatile and thought to more easily absorb into the lungs.	[26]

Table 5 Continued

Nicotine Absorption	An average of 4.5 mg of nicotine is absorbed from 7.9 g of chewing tobacco and an average of 3.6 mg of nicotine is absorbed from 2.5 g moist snuff.			[39]
Metabolites	Nornicotine and Cotinine			[37]
Conversion of nicotine to nornicotine	Among all alkaloids, nornicotine is less than 5 % present in tobacco. However, levels are increased by the metabolization of nicotine to nornicotine. CYP82E4 genes play a major role in the metabolic conversion of nicotine to nornicotine in tobacco. Nicotine is rapidly and extensively metabolized by the liver, primarily by the liver enzyme CYP2A6 [and to a lesser extent by CYP2B6 and CYP2E1] to cotinine			[6, 37, 39-41]
Level of alkaloids				
Nicotine	0.6–3.0 % of the dry weight of tobacco			[42]
	pH Nicotine		Nornicotine	Anabasine
Khani	9.47 0.53 - 21.3		-	-
Zarda	5.02 13.8 - 65		0.09	10.23
Gutkha	5.24 1.23-11.4		-	-
Mechanism of action				
Nicotine	Exerting reinforcing effects via activation of the nicotinic acetylcholine receptor [nAChR] in the mesolimbic dopamine system.			[37]
Anabasine and nornicotine	Elicit a calcium-dependent increase in striatal dopamine release through activation of the nAChR, and similarly to nicotine, produce nAChR desensitization			[43]
Effects				
Toxicity	Nicotine causes an increase in blood pressure, heart rate and also contributes to the hardening of arterial walls that ultimately leads to heart attack. Increased risk of CVS, GIT, RTI disorder Increase metabolic rate and physical activity while decreasing food intake, thus reduce body weight in user			[41]
Rodents	Nicotine, nornicotine, anatabine, and anabasine reduced food intake and motivation to initiate food intake, thus reducing body weight.			[44]

4.3 Carbonyls-aldehydes

Aldehydes are found in Smokeless Tobacco products as a result of fire-curing. The description of volatile carbonyls: Aldehydes [formaldehyde, crotonaldehyde, acetaldehyde] listed as HPHC by FDA are summarized in Table 6 and the mechanism is depicted in Figure 5.

Note: Fire-curing method: Involves hanging tobacco in a large enclosed area where it is exposed to smoke from hardwood fires that continuously burn or smolder. Used in the production of plug chewing tobacco, moist and dry snuff, and tobacco used to make iqmik. Causes chemical changes in the tobacco leaf, and contaminates the tobacco

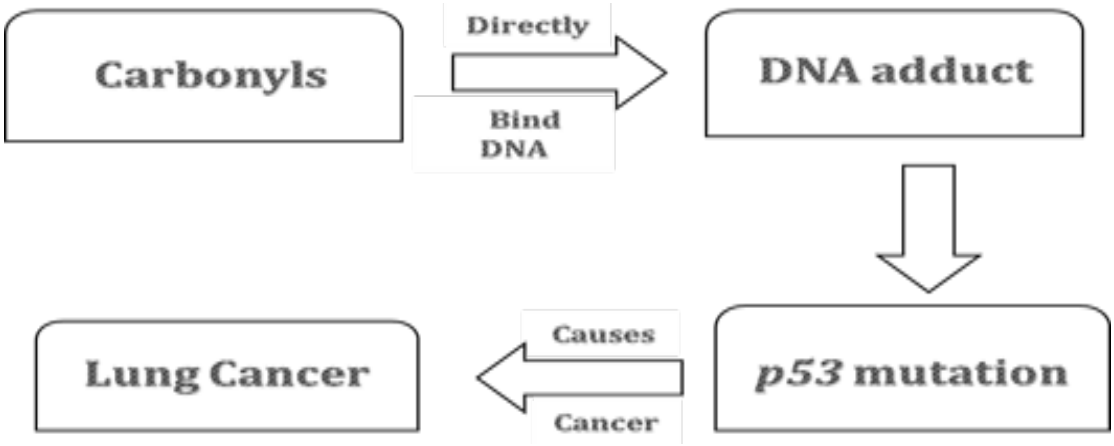


Figure 5. Illustrates Mechanism of Carbonyls Toxicity.
Carbonyls present in tobacco directly interact with DNA and form DNA adducts. Carbonyls-DNA adducts induce mainly G:C-to-T: A transversion mutations, the major type of mutations found in the p53 gene related to lung cancer.

Table 6. Description and effects of carbonyls

Data		References
Description		
Property	Carbonyls present in Smokeless Tobacco products are stable, extremely hydrophilic, and chemically reactive.	[45]
IARC Classification	Acetaldehyde: Group 2B Crotonaldehyde: Group 3 Formaldehyde: Group 1	[14, 46]
Nature	Acetaldehyde [Ethanal]: CA, RT, AD; Crotonaldehyde [2-Butenal]: CA; Formaldehyde [Methanal]: CA, RT	[16]
Level of Carbonyl		
Tobacco	10.5µg/g	[45]
Normal blood levels	In humans and animals, formaldehyde are approximately 2.5 ppm [2.5 mg/L]	[47]

Table 6 Continued

Effects		
Acetaldehyde	**Acute exposure:** Results in irritation of the eyes, skin, and respiratory tract. Anticipated to be a human carcinogen. On I.V.: addictive and enhances the addictiveness of nicotine in experimental animals **Large doses:** may cause death from respiratory paralysis associated with aldehyde dehydrogenase deficiency, which is an inborn error of metabolism	[48]
Crotonaldehyde	**In Animals:** dyspnea [breathing difficulty], pulmonary edema, irritation skin. *Rats:* Induces neoplastic lesions in the liver, hepatocellular carcinomas, neoplastic nodules, and liver damage when administered orally to rats over long periods of time. *Drosophila melanogaster*: Induction of sex-linked recessive lethal mutations **Human:** Sore throat, burning sensation behind the breastbone, cough, shortness of breath; In Skin: Redness. Burning sensation. Pain; watering of the eyes, nausea, abdominal pain, vomiting, diarrhea. Acute inhalational exposure leads to rapidly progressive acute respiratory distress syndrome [ARDS]. **High concentrations:** produce pulmonary edema **Mechanism of action:** It reacts with 2'-deoxyguanosine [dGuo] in DNA to generate a pair of diastereomeric adducts as alpha-methyl-gamma-hydroxy-1, [2]-propano-2'-deoxyguanosine [1,N[2]-propanodGuo] and Cro-dGuo. Cro-dGuo adducts inhibit DNA synthesis and induce miscoding in human cells.	[49, 50]
Formaldehyde	**Effects:** Neuro-toxicant; Effect on bone marrow cells and changes in the Prx2 expression Alters miRNA patterns that regulate gene expression potentially leading to the initiation of a variety of diseases. **Low concentration** increases calcium ion in cultured hippocampal neurons. **Mechanism of action:** Act via sensory nerve fibers that signal through the trigeminal nerve to reflexively induce bronchoconstriction through the vagus nerve. Induces mis-folding and aggregation of neuronal tau protein, hippocampal neuronal apoptosis, cognitive impairment and loss of memory functions, as well as excitation of peripheral nociceptive neurons in cancer pain models.	[47, 51-53]
Target organs	Eyes, skin, respiratory system	[47- 49]

Table 6 Continued

Toxicity	Induce mutagenic DNA damage Increase systemic oxidative stress and oxidative damage, contributing to the development of chronic diseases such as atherosclerosis, diabetes, and chronic obstructive pulmonary disease [COPD]. Induce carbonylation of proteins, which is an irreversible oxidative damage often associated with loss of protein function and accumulation of damaged or unfolded proteins Formaldehyde and crotonaldehyde significantly inhibited T-lymphocyte and B-lymphocyte proliferation	[55, 56]

with smoke-related chemicals such as polycyclic aromatic hydrocarbons, phenols, and volatile aldehydes.

4.4 Metals

Smokeless Tobacco products contain approximately 30 metallic compounds and toxicity reported to be directly

Table 7. Description and effects of metals

Data		References
Description		
About	Trivalent arsenic [As] is more toxic than pentavalent forms Beryllium [Be] naturally found in mineral rocks, coal, soil, and volcanic dust. Among all metals present in tobacco products, cadmium [Cd] concentration is highest. Metal is found in earth crust. Chromium [Cr] is naturally occurring metal in different forms. Its concentration is high in all five lobes of smokers. Nickel [Ni] is the 24th most abundant, naturally occurring element. Lead [Pb] is a naturally occurring element and corrosion resistant. Mercury [Hg] naturally occurs in environment, usually in form of mercury sulfide Selenium [Se] is a biologically essential trace element as well as an antioxidant enzyme.	[58-66]
IARC Classification	As: Group 1 Be: Group 1 Cd: Group 1 Cr: Group 3 Hg: Group 3 Ni: Group 2B Pb: Group 2A Se: Group 3	[14]

Table 7 Continued

Nature	As: CA, CT, RDT Be: CA Cd: CA, RT, RDT Cr: CA, RT, RDT Hg: CA, RDT Ni: CA, RT Pb: CA, CT, RDT Se: RT	[16]
Level of metals		
Tobacco [per gram]	As: 130 - 360 ng/g [0.8 to 2.4 micrograms of inorganic arsenic per pack of 20 cigarettes] Cd: 0.1-3.1µg/g Cr: 5.25-21.9µg/g Ni: 1.33-13.1µg/g Pb: 1.76-13µg/g	[66, 67]
flue-cured tobacco	As: 400 ng/g	[67]
Daily maximum acceptable level	As:15 µg/kg Cd: 0.5 µg/kg Ni: 0.5 mg/kg Pb: 3.57 µg/kg	[36, 68]
PTWI [µg/kg/ week]	As: 15 Cd: 3.5 Pb: 25 Hg: 4	[36, 68]
Per day Intake [µg/kg/day]	As: 3 Cd: 0.2-1 Pb: 5	[36, 68]
For a 60 kg Individual [µg/day]	As: 180 Cd: 30 Pb:300	[36, 68]
Exposure	As: In tobacco farming, Arsenic containing pesticides are used that remain in tobacco Be: Occupational exposure and tobacco consumption [oral and inhalation] Cd: Through soil in tobacco plants, Smoking [inhalation], occupational Cr: Dietary exposure Hg: Dietary Exposure Ni: Oral and Ingestion Pb: Dietary exposure, drinking water and inhalation Se: Occupational, Hazardous waste site	[58-65]

Table 7 Continued

Effects		
Arsenic	**Acute poisoning:** abdominal pain, nausea and vomiting, diarrhea, muscle cramps, metallic taste and extreme thirst, followed by stupor, coma, cardiovascular collapse and death **Chronic poisoning:** Arsenosis and skin manifestations **Humans:** Cancer [liver, lung, skin], skin lesions, developmental effects, CVS, neurotoxicity and diabetes. Chronic Exposure: Liver injury Enhance cancer i.e. act as **co-carcinogens** that activate other chemical compounds **Symptoms**: Low hemoglobin [blood cells production decreased], acute renal failure [renal insufficiency], nerve damages [painful neuropathy], mouth ulceration, needle sensation in hands and feet [rash], GIT irritation, conjunctival and respiratory tract inflammation, epistaxis. **Carcinogenic Mode of action**: Involve cytotoxicity to the bladder epithelium and sustained increased cell proliferation. **Target Organ:** Dermal [Skin], GIT [Digestive], Hepatic [Liver], Neurological [Nervous System], Respiratory [From the Nose to the Lungs]	[58, 67, 69-72]
Beryllium	**Animals:** Gene mutation, Teratogenic effect, reproductive, carcinogenic [pulmonary adenomas] **Dogs:** GIT lesions and bone marrow hypoplasia Rats: Rickets **Humans:** Contact dermatitis, lung cancer, osteosarcoma; acute or chronic beryllium disease [ABD/CBD]: acute respiratory disease, granulomatous interstitial pneumonitis. **Symptoms:** Lung [pneumonitis, fibrosis, proliferative lesions, metaplasia and hyperplasia]; Berylliosis[chronic exposure]: anorexia, weight loss, lassitude [weakness, exhaustion], chest pain, cough, clubbing of fingers, cyanosis, pulmonary insufficiency; irritation eyes. **Mechanism of Action:** Interfere with nucleic acid metabolism on transcriptional level; thus, inhibit replication of DNA in liver. Secondly lung toxicity might be through hypersensitization or direct toxicity as in CBD disease, wherein on inflammation response- phagocytosis leads to subsequent release of lysosomal enzymes consequently damaging the lungs **Target Organ:** GIT and skeletal system [oral]; Lung [inhalation]	[59, 73, 74]

Table 7 Continued

Cadmium	**Short-term:** Respiratory system irritation [throat and mucosa], After lag: influenza-like symptoms including cough, tight chest, pain in chest on coughing and dyspnoea emerged. Beyond 8 h wheezing, chest pain, precordial constriction and persistent cough. **Long-term:** emphysema, COPD, death from bronchitis, **Signs and symptoms:**Causes renal failure, Pancreatic cancer, placental necrosis, hypertension, anemia, hepatic damage, lung damage, osteomalacia, testicular tumors, pulmonary edema, hypertension and induce deficiencies of other essential minerals like iron, zinc, and copper. Cd is highly toxic to bones, nervous systems and can accumulate in the lenses of the eyes and cause cataracts. **Mode of action:** Causes kidney damage because Cd competes with zinc for biological binding sites and ultimately affects the kidney. Lung damage is caused by altered redox balance [oxidative stress and making macrophages dysfunctional. FDA has set 55 μg as the tolerable daily intake of cadmium per individual.	[60, 75-79]
Chromium	**Conversion:** Chromium is present in tobacco in its oxidation state [Chromium III], manganese oxides is responsible to oxidize chromium [III] to chromium [VI] **Signs and symptoms:** Respiratory impairment- Irritation of the lining of the nose, runny nose, and breathing problems [asthma, cough, shortness of breath, wheezing], oral and epidermal allergic contact dermatitis, pulmonary sensitization, hemorrhage, and liver lesions in humans **Animals:** Irritation and ulcer in stomach, anemia, sperm damage **Mechanism of action:** The toxic potency of chromium is dependent on the oxidation state of the chromium atom, with chromium[VI] more potent than chromium[III]. The mechanisms are mediated partly through reactive intermediates during intracellular reduction of chromium[VI] to chromium[III] and oxidative reactions, and partly mediated by Chromium[III] that may form complexes with peptides, proteins, and DNA, resulting in DNA-protein crosslinks, DNA strand breaks, and alterations in cellular signaling pathways, which may contribute to toxicity and carcinogenicity of chromium compounds. Location of particle deposition in the lung and extracellular dissolution of chromium[VI] compounds is also considered a mechanism to cancer. **Target Organs**: Immunological [Immune System], Renal [Urinary System or Kidneys], Respiratory [From the Nose to the Lungs]	[61, 80-82]

Table 7 Continued

Mercury	**Rats** [single dose]: Hematological, hepatic, renal effects **Humans:** Renal effects: interstitial sclerosis, renal tubular damage and proximal tubular necrosis **High doses:** Severe gastrointestinal damage as inflammation and necrosis of the forestomach and necrosis of the glandular stomach. **General Toxicity [Long term]:** Decrease in body weight gain, changes in clinical and hematological parameters, as well as organ-specific effects [increased kidney and adrenal weights, testicular atrophy. Neurotoxicity is observed with methyl mercury. **Genotoxic Effects:** Single-stranded DNA breaks, sister chromatid exchanges and chromosomal aberrations. **Mechanism of action:** Induction of oxidative stress [reactive oxygen species] or disruption of microtubules rather than direct interaction with DNA, including adduct formation **Target Organs:** Nervous system, hepatic system and renal system	[62, 68, 83-85]
Nickel	**Animals:** Lung tumors, reproductive and developmental effects **Signs and symptoms in Humans** **Acute**: pulmonary fibrosis and inflammation, stomach aches, blood [increased red blood cells], kidneys [increased protein in the urine, edema]. GIT distress. **Chronic:** Inflammatory responses, such as allergy on contact, rashes, skin sensitization [dermatitis]. The chronic irritation-induced inflammation of epithelial tissue is associated with the risk of neoplastic changes. On inhaling, chronic bronchitis, reduced lung function, cancer of the lung and nasal sinus, pneumoconiosis and asthma can be observed. **Mechanism of action:** Promote cell proliferation, which would convert repairable DNA lesions into non-repairable mutations. Nickel sensitization induced inflammation occurs via binding to the copper-binding site of human serum albumin and sensitization to the resulting metal–protein complex. **Target organs:** Skin and Lungs	[63, 66, 68, 72, 86, 87]
Lead	**Animals:** Affects Heart, bones, intestines, kidneys, and reproductive and nervous systems. It mainly affects the haematopoietic system. **Humans:** Exposure to lead affects numerous organs systems, primarily liver, kidney and brain. **Children**: highly dangerous because of its toxic effect on brain and responsible for intellectual impairment and behavioural disorders [poor learning outcomes and decreased intelligence quotient level] as well as impact on hematic and immune systems. Anaemia and gum infection among children was often an indicator of lead poisoning.	[64, 77, 84, 85, 88-91]

Table 7 Continued

Lead	**Adults**: increase in blood pressure, suppress cognitive development, increase risk of cardiovascular diseases and negatively impact on male reproductive system. Chronic exposure to lead, damages the brain and leads to convulsions, coma or even death. **Mechanism of action:** Oxidative stress plays a role in lead-induced toxicity, lead has been shown to deplete antioxidant proteins and induce the production of Reactive Oxygen Species [ROS]. Lead can cause toxicity in several ways: It directly interferes with enzyme activation, causes competitive inhibition of trace mineral absorption, binds with sulfhydryl proteins, alters calcium homeostasis and lowers the level of sulfhydryl antioxidant reserves in the body. **Target organs:** Lead poisoning was found to be often associated with encephalopathy and toxic neuropathy suggesting the brain as the most vulnerable target.	[64, 77, 84, 85, 88-91]
Selenium	**Animals:** Affect female reproductive system in rats and changes in menstrual cycle in monkey. Selenium salts and acids have effects on cardiovascular, gastrointestinal, hematological, hepatic, dermal, immunological, neurological, and reproductive systems. **Humans:** **Signs and symptoms:** On skin contact: cause rashes, redness, heat, swelling, pain. On ingestion, dizziness, fatigue, and irritation of mucous membranes is observed. Stomach ache and headache is also reported. **Chronic:** Collection of fluid in the lungs [pulmonary edema] and severe bronchitis have been reported, brittle hair and deformed nails can develop, loss of feeling and control in arms and legs. Selenosis disease affects the dermal and neurological system including unsteady gait and paralysis. On the contrary, selenium is less than required by the body, heart problems and muscle pain would occur. Specifically known as keshan disease [cardiomyopathy] and Kashin-beck disease [osteoarthropathy]. **Mechanism of Action:** Selenium readily substitutes for sulfur in biomolecules [certain amino acids, including the disulfide bridges that provide tertiary structure] and in many biochemical reactions, especially. Inactivation of the sulfhydryl enzymes necessary for oxidative reactions in cellular respiration, through effects on mitochondrial and microsomal electron transport, contribute to toxicity. Selenium status can also influence thyroid hormone function via the deiodinase enzymes. **Target organs**: Lung and Liver	[65, 84, 92]

proportional to the disease burden in our body[57]. The description of trace metals issummarized in Table 7.

4.5 Nitrosamines

Smokeless Tobacco products consist of Tobacco-specific Nitrosamines [TSNA] as well as some other Nitrosamines [NA] which are known prominent carcinogens. The description is summarized in Table 8 and mechanism is illustrated in Figure 6.

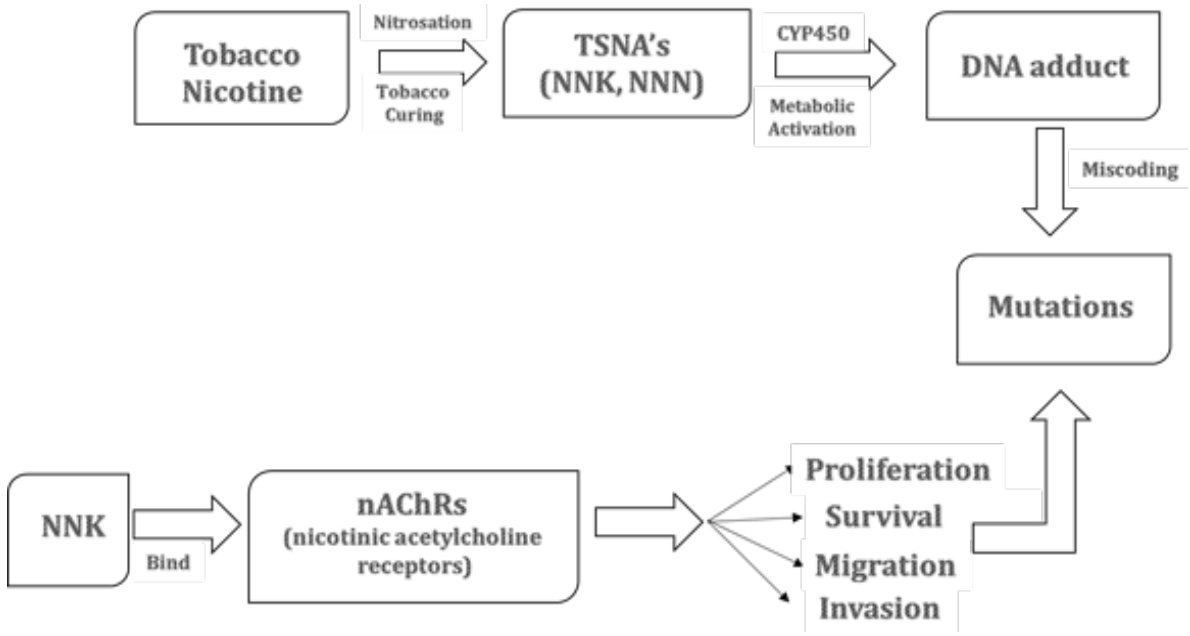


Figure 6. Illustrates Mechanism of Nitrosamines Toxicity.
Nitrosamines [NNK and NNN] mechanism induces carcinogenic processes in two different aspects. Nitrosamines metabolized by cytochrome P450 [CYP450] results in the formation of DNA adducts. Unresolved DNA adducts further cause mutations of tumor suppressor genes. Another mechanism is the binding of NNK and NNN to nAChRs [nicotinic acetylcholine receptors] that promote tumor growth by enhancing and deregulating cell proliferation, cell survival and cell migration as well as cell invasion. Both these aspects of nitrosamines induce cancer in tobacco users.

Table 8. Description and effects of Nitrosaminesi

Data		References
Description		
About	Nitrosamines are a group of organic chemicals formed by the interaction of nitrites with amines or amides TSNA's are nicotine derived [in other words N-nitroso-derivatives of pyridine-alkaloids]	[93]

Table 8 Continued

TSNA's	1. N.nitrosonornicotine [NNN] 2. N'-nitrosoanatabine [NAT] 3. N'-nitrosoanabasine [NAB] 4. 4-[methylnitrosamino]-1-[3-pyridyl]-1-butanone [NNK] Metabolite of NNK is 4-[methylnitrosamino]-1-[3-pyridyl]-1-butanol [NNAL] NNN, NNK, NNAL: occur in quantities greater than other nitrosamines	[94]
Other NA's	Volatile N-nitrosamines 1. N-Nitrosodimethylamine[NDMA] 2. N-Nitrosomorpholine[NMOR] 3. N-Nitrosopiperidine[NPIP] 4. N-Nitrosopyrrolidine[NPYR] *Volatile N- Nitrosoalkalonamine* is 5. N-Nitrosodiethanolamine[NDELA] Non-volatile N-nitrosamino acids 6. N-Nitrososarcosine[NSAR]	[95]
IARC Classification	NNK: Group 1 NNN: Group 1 NDMA: Group 2A NMOR: Group 2B NPIP: Group 2B NPYR: Group 2B NSAR: Group 2B NDELA: Group 2B	[14]
Nature	NNK, NNN, NDMA, NMOR, NPIP, NPYR, NSAR, NDELA- CA	[16]
Level of Nitrosamines		
Tobacco [per gram]	3.3µg/g	[96]
flue-cured tobacco	127.93µg/g	
High concentration	Khaini [Indian brand]	

Table 8 Continued

Formation of Nitrosamines		
Conversion	TSNAs are naturally present in fresh green tobacco [formation from nitrates in soil] and increases [formation from nicotine] during curing, storage, fermentation, and processing of harvested tobacco leaves. TSNAs [NNN, NNK, NAT and NAB] are formed by nitrosation reaction of alkaloids during curing and processing of tobacco. Being derived from nicotine, and therefore are associated only with tobacco, tobacco smoke and other nicotine-containing products. TSNA's as well as their metabolites and macromolecular adducts are ideal markers for assessing human exposure to, and metabolic activation of, tobacco smoke carcinogens. NDELA is formed from diethanolamine, a residual contaminant in tobacco.	[94-97]
Effects		
Toxicity	**Animals**: TSNA's and VNA's induce cholangiocarcinoma [CCC- bile duct cancer]. **NDMA**: liver carcinogenic in mice, rats, hamsters, as well as in rabbits and mink. Increase in coagulation time, prothrombin time, plasma cholinesterase levels and leukopenia in dogs with hemorrhage in liver and abdominal tissues. Reddening of eyes and piloerection in rats. **NDELA**: hepatocarcinogenic [HCC] in hamsters and rats **NMOR**: Hepato-carcinogenesis, tumor promoting effects and cytotoxicity with signs as decrease in epinephrine levels and no. of liver foci **NPIP:** Bronchogenic and alveologenic tumors in mice; Mutagenicity and apoptosis in rodents and hamsters. Esophageal hyperplasia observed. Induction of liver tumors. **NPYR:** On exposure during pregnancy, neoplasm to the offspring in rats. Induce tumors in the liver but not lungs.	

Table 8 Continued

Toxicity	**NSAR:** Nasal, esophageal and liver cancer **NNK**: Induction of tumor in lung, liver, nasal cavity, pancreas in rats, **NNAL**: induce lung tumors **NNN:** Esophageal carcinogen, lung tumors and the upper aerodigestive tract of mice, rats, hamsters and mink.	[3, 97-111]
	Humans: TSNA's causes cancers of the esophagus, nasal cavity, and pancreas. Cancer of the lung and of the upper aerodigestive tract. **NDELA:** Irritates the skin and eyes and may damage the liver and kidneys **NDMA:** Hepatotoxicity is a predominant effect. Causes hemorrhages in the bronchi, trachea, GIT, sub-pericardial hemorrhage. On inhalation exhaustion, headache, abdominal cramps, soreness on the left side, nausea, vomiting and may lead to jaundice and ascites. On chronic exposure probability of tumors in lung, liver and kidney. **NMOR:** Gene mutagenicity [DNA lesion, chromosomal aberrations], carcinogenicity [liver and kidney], cyanosis, hypoxia.	
	NPIP: Carcinogenic agent [throat, liver and lung and stomach], mutagen [oxidative DNA damage], apoptosis inducer. Signs are irritation of eyes, nose, throat and skin. Inflammation to pigmented area, damage to cornea, reduced vision **NPYR:** Irritates the skin, eyes and can damage the liver and kidneys. Causes HCC. **NSAR:** Contact can irritate the skin and eyes. Reproductive damages **NNN and NNK**: Induce 2 types of primary DNA lesions: nucleotide methylations and pyridyloxo-butylations [HPB adducts]	
Mechanism of toxicity	**General:** Induces free radical production, leading to the formation of mutagens, DNA lesions and cellular proliferation of the intrahepatic bile duct epithelium **Specific:** NDMA: alkylation of cellular protein; NMOR: increase the rate of genetic mutation by interfering with the function of nucleic acids; NPIP: alpha-hydroxylation leads to mutagenicity; NPYR: alpha-hydroxylation and oxidative stress.	
Target Organ	Liver, lungs and kidney	

4.6 Polynuclear aromatic hydrocarbons:

PAHs are a class of compounds composed of two or more fused benzenoid rings known for their carcinogenic and mutagenic properties. There are more than a hundred different PAHs identified in tobacco[112]. The description of PAHs is summarized in Table 9 and the mechanism is illustrated in Figure 7.

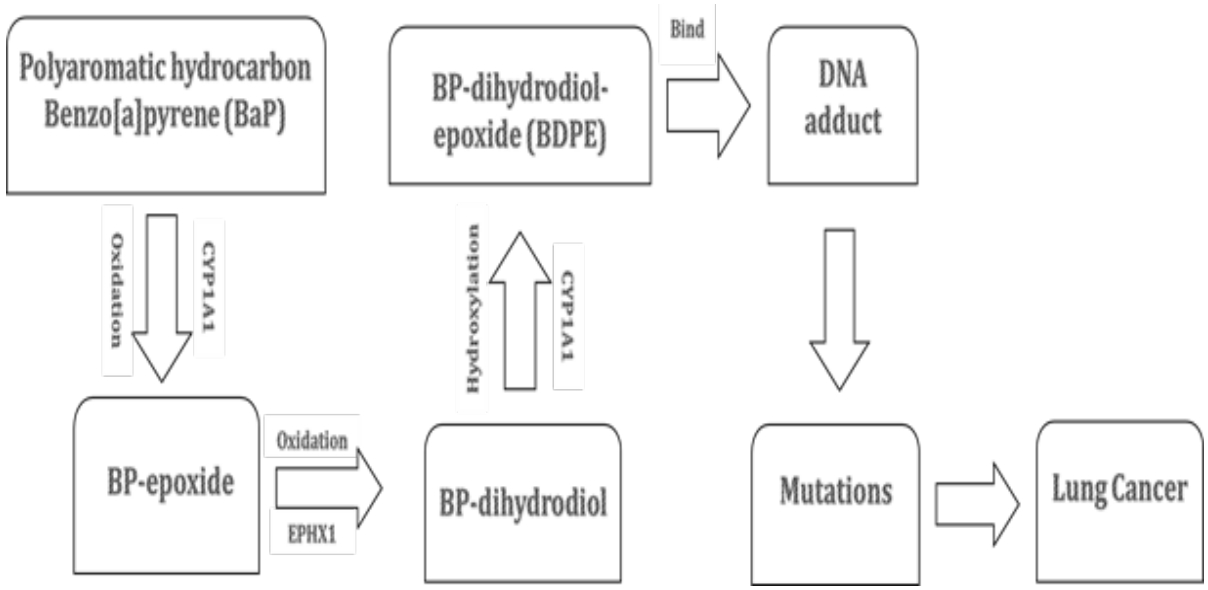


Figure 7. Illustrates Mechanism of Polyaromatic hydrocarbons Toxicity.
Most common polyaromatic hydrocarbon BaP and dibenzanthracene is transformed into BP-7,8-epoxide by CYP1A1[AHH [aryl hydrocarbon hydroxylase] Cytochrome P450, family 1, subfamily A, polypeptide 1]. Epoxide hydrolases further oxidized BP-7,8-epoxide to form BP-7,8-dihydrodiol. In the final step CYP1A1-catalyzed hydroxylation to form BP-7,8-dihydrodiol-9,10-epoxide [BPDE], the ultimate carcinogen. On the p53 gene, mutation of the sequences leads to carcinogenesis linked to human lung cancer.

Table 9. Description of Polynuclear Aromatic Hydrocarbons [PAHs]

Data		References
Description		
About	PAHs are groups of chemicals that generally occur as complex mixtures. PAHs do not occur naturally in plant material, the curing process can introduce PAHs to the leaf if the tobacco is exposed to exhaust gases from heat sources that rely on burning wood or other organic fuels [known as fire-cured tobacco varieties].	[112, 113]

Table 9 Continued

IARC Classification	Naphthalene Benzo[a] anthracene Benzo[a] pyrene Benzo[b] fluoranthene Benzo[k] fluoranthene Chrysene Indeno[1,2,3-cd] pyrene Dibenz[a,h] anthracene	NAP BAA BAP BBF BKF CHR IPYR DBAA	Group 2B Group 2B Group 1 Group 2B Group 2B Group 2B Group 2B Group 2A	[14]
Nature	NAP BAA CHR BBF BKF BAP IPYR DBAA	CA, RT CA, CT CA, CT CA, CT CA, CT CA CA CA		[16]
Level and sources of Polynuclear aromatic hydrocarbons				
Tobacco	0.94 µg/g			[96]
Exposure	During fire curing of SLT, Charred tobacco use or smoking			[113]
Effects				
Symptoms/ Toxicity in Humans	Respiratory system: Bloody vomit, breathing problems, chest pains, chest and throat irritation, and abnormalities in chest X-rays have been reported. CVS: Arterial smooth muscle cell proliferation, cell necrosis, collagen synthesis, lipid accumulation [atherosclerotic plaque]. GIT: melanosis of colon and rectum. Skin: chronic dermatitis, hyperkeratosis, skin ulcerations, hyperplasia, and hyperkeratosis. Death due to adverse hematological effects [e.g., aplastic anemia and pancytopenia resulting in hemorrhage], bone marrow toxicity. May induce autoimmune responses, alter levels of neurotransmitters, eye irritation, nausea and vomiting. High exposure of PAH causes cancer. Lung tumors are observed upon local administration or inhalation			[112 -118]

Table 9 Continued

Animals	Harmful effects on the skin, body fluids, and ability to fight disease after both short- and long-term exposure. Epithelial hyperplasia, preneoplastic hepatocytes, dilated tubules are observed. Cancer of Lung, stomach and skin on eating and being in contact. *Mice:* On feed during pregnancy causes difficulty reproducing and so did their offspring. The offspring also had higher rates of birth defects and lower body weights	
Target organ	Respiratory system, liver and kidney	[113]

5. Conclusion

This review emphasizes the significant association of Smokeless Tobacco product components with health hazards. Pathways estimates for smokeless tobacco-associated disease need further analysis. Profiling of Smokeless Tobacco products also requires multi-centric well-designed studies. Further, the information would be a guide for researchers interested in components of SLT products.

6. Acknowledgements

The authors are grateful to the team of HRRIC [Vikas Punamiya, Pulmonologist- Advisor; Shweta Vani, PMO; and Francie Patel, COO] for their overall support and encouragement in addition to assistance in reviewing this article.

7. Conflict of Interests

The authors declare that there is no conflict of interest in the publication.

8. References

1. Thakur JS, Paika R. Determinants of Smokeless Tobacco use in India. Indian J Med Res. 2018; 148:41–5. PMid: 30264753 PMCid: PMC6172920. https://doi.org/10.4103/ijmr.IJMR_27_18.
2. IARC [International Agency for Research on Cancer]. Betel-quid and areca-nut chewing and some areca-nut-derived nitrosamines, IARC monographs on the evaluation of carcinogenic risks to humans, Lyon, France; 2004; 85. 2020. https://publications.iarc.fr/103.
3. SCENIHR [Scientific Committee on Emerging and Newly Identified Health Risks]. Scientific opinion on Health effects of Smokeless Tobacco products, European Commission, 2008. 2020. http://ec.europa.eu/health/archive/ph_risk/committees/04_scenihr/docs/scenihr_o_013.pdf.
4. Stanfill SB, Connolly GN, Zhang L, Jia LT, Henningfield JE, Richter P, Lawler TS, Yusuf OA, Ashley DL, Watson CH. Global surveillance of oral tobacco products: Total nicotine, unionised nicotine and tobacco-specific N-nitrosamines. Tob Control. 2011; 20(3):e2. PMid: 21109685. https://doi.org/10.1136/tc.2010.037465.
5. IARC [International Agency for Research on Cancer]. Smokeless Tobacco and some tobacco-specific N-nitrosamines, IARC monographs on the evaluation of carcinogenic risks to humans, Lyon, France. 2007; 89. 2020. http://monographs.iarc.fr/ENG/Monographs/vol89/index.php.
6. Moghbel N, Ryu B, Ratsch A, Steadman KJ. Nicotine alkaloid levels and nicotine to nornicotine conversion in Australian Nicotiana species used as chewing tobacco. Heliyon 2017; 3:e00469. PMid: 29264422 PMCid: PMC5727613. https://doi.org/10.1016/j.heliyon.2017.e00469.
7. Yuan JM, Gao YT, Wang R., Chen, M, Carmella SG, Hecht SS. Urinary levels of volatile organic carcinogen and toxicant biomarkers in relation to lung cancer development in smokers. Carcinogenesis. 2012; 33:804–9. PMid: 22298640

PMCid: PMC3384073. https://doi.org/10.1093/carcin/bgs026.

8. Krusemann E, Visser WF, Cremers JWJM, Pennings JLA, Talhout R. Identification of flavour additives in tobacco products to develop a flavour library. Tob Control. 2017; 27(1):105–11. PMid: 28190004 PMCid: PMC5801651. https://doi.org/10.1136/tobaccocontrol-2016-052961.
9. Lisko JG, Stanfill SB, Watson CH. Quantitation of ten flavor compounds in unburned tobacco products. Anal Method. 2014; 6(13):4698–704. PMid: 26388954 PMCid: PMC4575278. https://doi.org/10.1039/C4AY00271G.
10. Pickworth WB, Rosenberry ZR, Gold W, Koszowski BJ. Nicotine absorption from Smokeless Tobacco modified to adjust pH. Addict Res Ther. 2014; 5:184. PMid: 25530912 PMCid: PMC4271311. https://doi.org/10.4172/2155-6105.1000184.
11. Zitomer N, Rybak ME, Li Z, Walters MJ, Holman MR. Determination of Aflatoxin B1 in Smokeless Tobacco products by use of UHPLC-MS/MS. J Agric Food Chem. 2015; 63(41):9131–8. PMid: 26452144 PMCid: PMC5697909. https://doi.org/10.1021/acs.jafc.5b02622.
12. Knipstein B, Huang J, Barr E, Sossenheimer P, Dietzen D, Egner PA, *et al*. Dietary aflatoxin-induced stunting in a novel rat model: Evidence for toxin-induced liver injury and hepatic growth hormone resistance. Pediatr Res. 2015; 78(2):120–7. PMid: 25938735 PMCid: PMC4506701. https://doi.org/10.1038/pr.2015.84.
13. Lane KS. Aflatoxin, tobacco, ammonia and the p53 tumor-suppressor gene: Cancer's Missing Link? Med Gen Med 1999; 1(2):E10. https://pdfs.semanticscholar.org/81cb/0a4d7ad80275ec471243e1a3dcabccf15b9f.pdf.
14. IARC [International Agency for Research on Cancer]. Agents classified by the IARC monographs, 2019; 1–123. 2020. http://monographs.iarc.fr/ENG/Classification/ClassificationsAlphaOrder.pdf.
15. National Center for Biotechnology Information. PubChem Database. Aflatoxin B1, CID=186907. 2020. https://pubchem.ncbi.nlm.nih.gov/compound/Aflatoxin-B1.
16. FDA. Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke, Established List. U.S. Food and Drug Administration, U.S. Department of Health and Human Services. 2012. 2020. http://www.fda.gov/downloads/TobaccoProducts/GuidanceComplianceRegulatoryInformation/UCM297981.pdf.
17. WHO [World Health Organisation]. Food safety digest-Aflatoxins. Department of Food Safety and Zoonoses. 2018. 2020. https://www.who.int/foodsafety/FSDigest_Aflatoxins_EN.pdf.
18. FDA [Food and Drug Administration]. Compliance Policy Guide Sec. 638.100. Action Levels for Aflatoxins in Animal Food. 2019. 2020. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cpg-sec-683100-action-levels-aflatoxins-animal-feeds.
19. Inaba Y, Uchiyama S, Kunugita N. Spectrophotometric determination of ammonia levels in tobacco fillers of and sidestream smoke from different cigarette brands in Japan. Environ Health Prev Med. 2018: 23(15). PMid: 29703135 PMCid: PMC5923008. https://doi.org/10.1186/s12199-018-0704-5.
20. Padappayil RP, Borger J. Ammonia toxicity. Stat Pearls [Internet], Treasure Island [FL]: Stat Pearls Publishing. 2020 [Updated 2019 Oct 26]. 2020. https://www.ncbi.nlm.nih.gov/books/NBK546677/.
21. Stevenson T, Proctor RN Am J. Phillip Morris and the origins, spread and denial of nicotine freebasing. Public Health. 2008; 98(7):1184–94. PMid: 18511721 PMCid: PMC2424107. https://doi.org/10.2105/AJPH.2007.121657.
22. Watson CV, Blasini LV, Damian M, Watson CH. Method for the determination of ammonium in cigarette tobacco using ion chromatography. Regul Toxicol Pharmacol. 2015; 72:266. PMid: 25934256 PMCid: PMC5712451. https://doi.org/10.1016/j.yrtph.2015.04.019.
23. EPA [Environmental Protection Agency]. Toxicological Review of Ammonia [Noncancer Inhalation]. 2016. 2020. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0422tr.pdf.
24. Agency for Toxic Substances and Disease Registry [ATSDR]. Toxicological profile for Ammonia. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. 2004. 2020. https://www.atsdr.cdc.gov/toxprofiles/tp126.pdf.
25. van Amsterdam J, Sleijffers A, van Spiegel P, Blom R, Witte M, van de Kassteele J, *et al*. Effect of ammonia in cigarette tobacco on nicotine absorption in human smokers. Food ChemToxico.l 2011; 49(12):3025–30. PMid: 22001171. https://doi.org/10.1016/j.fct.2011.09.037.
26. Jeong-Im J. Food and Drug Administration Centre for Tobacco Product. 2015. 2020. https://www.fda.gov/media/124668/download.
27. Bhisey RA. Chemistry and toxicology of Smokeless Tobacco. Indian J Cancer. 2012; 49(4):364–72. PMid: 23442400. https://doi.org/10.4103/0019-509X.107735.
28. McAdam K, Enos T, Goss C, Kimpton H, Faizi A, Edwards S, *et al*. Chem Cent J. 2018; 121:142. http://dx.doi.org/10.1186/s13065-018-0506-2.
29. Lake BG. Coumarin metabolism, toxicity and carcinogenicity: Relevance for human risk assessment. Food Chem

Toxicol. 1999; 37(4):423–53. https://doi.org/10.1016/S0278-6915(99)00010-1.

30. EFSA [European Food Safety Authority]. Coumarin in flavourings and other food ingredients with flavouring properties. Scientific opinion of the panel on food additives, flavourings, processing aids and materials in contact with food [AFC]. EFSA J 2008; 793:1–15. 2020. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2008.793.
31. McAdam K, Vas C, Kimpton H, Faizi A, Liu C, Porter A, *et al*. Ethyl carbamate in Swedish and American Smokeless Tobacco products and some factors affecting its concentration. Chem Cent J. 2018; 12(1):86. PMid: 30043180 PMCid: PMC6057859. https://doi.org/10.1186/s13065-018-0454-x.
32. EPA [Environmental Protection Agency]. Initial List of Hazardous Air Pollutants with Modifications. 2017. 2020. https://www.epa.gov/haps/initial-list-hazardous-air-pollutants-modifications#mods.
33. Dinesh TK, Gourishankar S, Mehta SK. Quantification of ethyl carbamate in tobacco. 2012. 2020. https://www.coresta.org/sites/default/files/abstracts/2012_TSRC15_Mehta.pdf.
34. Jiao Z, Dong Y, Chen Q. Ethyl carbamate in fermented beverages: Presence, analytical chemistry, formation mechanism and mitigation proposals. Compr Rev Food Sci F. 2014; 13(4):611–26. PMid: 33412714. https://doi.org/10.1111/1541-4337.12084.
35. IARC [International Agency for Research on Cancer]. Alcohol consumption and ethyl carbamate. In: IARC monographs on the evaluation of carcinogenic risks to humans. 2010. 2020. https://monographs.iarc.fr/wp-content/uploads/2018/06/mono96.pdf.
36. FAO, 64th Joint FAO/WHO Expert Committee on Food Additives [JECFA] meeting - Food contaminants. Summary and conclusions, 2005; p. 1–47. 2020. http://www.fao.org/3/a-at877e.pdf.
37. Benowitz NL. Pharmacology of nicotine: Addiction, smoking-induced disease and therapeutic. Annu Rev Pharmacol Toxicol. 2009; 49:57–71. PMid: 18834313 PMCid: PMC2946180. https://doi.org/10.1146/annurev.pharmtox.48.113006.094742.
38. Zhang J, Ji H, Sun S, Mao D, Liu H, Guo Y. Selective determination of pyridine alkaloids in tobacco by PFTBA ions/analyte molecule reaction ionization ion trap mass spectrometry. J Am Soc Mass Spectrom. 2007; 18(10):1774–82. PMid: 17716908. https://doi.org/10.1016/j.jasms.2007.07.017.
39. Severson HH. What have we learned from 20 years of research on Smokeless Tobacco cessation? Am J Med Sci. 2003; 326(4):206–11. PMid: 14557736. https://doi.org/10.1097/00000441-200310000-00011.
40. Siminszky B, Gavilano L, Bowen SW, Dewey RE. Conversion of nicotine to nornicotine in Nicotianatabacum is mediated by CYP82E4, a cytochrome P450 monooxygenase. Proc Natl Acad Sci. 2005; 102:14919–24. PMid: 16192354 PMCid: PMC1253577. https://doi.org/10.1073/pnas.0506581102 .
41. National Center for Biotechnology Information. PubChem Database. Nicotine, CID=89594, 2020. https://pubchem.ncbi.nlm.nih.gov/compound/Nicotine.
42. Rai SK, Tewari AK. In synthesis of medicinal agents from plants. Elsevier. 2018; 305–32. PMid: 29291582. https://doi.org/10.1016/B978-0-08-102071-5.00013-1.
43. Clemens KJ, Caillé S, Stinus L, Cador M. The addition of five minor tobacco alkaloids increases nicotine-induced hyperactivity, sensitization and intravenous self-administration in rats. Int J Neuropsychopharmacol. 2009; 12(10):1355–66. PMid: 19366487. https://doi.org/10.1017/S1461145709000273.
44. Bunney PE, Hansen M, LeSage M. Effects of isolated tobacco alkaloids and tobacco products on deprivation-induced food intake and meal patterns in rats. Pharmacol Biochem Behav 2018; 165:45–55. PMid: 29196096 PMCid: PMC5801111. https://doi.org/10.1016/j.pbb.2017.11.004.
45. Coresta. Technical report. Carbonyls-in-Tobacco-and-Tobacco-Products. 2017. 2020. https://www.coresta.org/sites/default/files/technical_documents/main/TTPA-043-1-CTR_Carbonyls-in-Tobacco-and-Tobacco-Products_Jan2018.
46. Kaur J, Sharma A, Kumar A, Bhartiya D, Sinha D, Kumari S, *et al*. SLTChemDB: A database of chemical compounds present in Smokeless Tobacco products. Sci Rep. 2019; 9:7142. PMid: 31073139 PMCid: PMC6509116. https://doi.org/10.1038/s41598-019-43559-y.
47. National Center for Biotechnology Information. PubChem Database. Formaldehyde, CID=712. 2020. https://pubchem.ncbi.nlm.nih.gov/compound/Formaldehyde.
48. National Center for Biotechnology Information. PubChem Database. Acetaldehyde, CID=177. 2020. https://pubchem.ncbi.nlm.nih.gov/compound/Acetaldehyde.
49. National Center for Biotechnology Information. PubChem Database. Crotonaldehyde, CID=447466. 2020. https://pubchem.ncbi.nlm.nih.gov/compound/Crotonaldehyde.
50. Huang JF, Zhu DM, Ma JF, Zhong M. Acute respiratory distress syndrome due to exposure to high-concentration mixture of ethene and crotonaldehyde. ToxicolInd Health. 2015; 31(7):585–7. PMid: 23448861. https://doi.org/10.1177/0748233713480205 .

51. Chi YN, Zhang X, Cai J, Liu FY, Xing GG, Wan Y. Formaldehyde increases intracellular calcium concentration in primary cultured hippocampal neurons partly through NMDA receptors and T-type calcium channels. Neurosci Bull. 2012; 28(6):715–22. PMid: 23160928 PMCid: PMC5561821. https://doi.org/10.1007/s12264-012-1284-9 .
52. Rager JE, Smeester L, Jaspers I, Sexton KG, Fry RC. Epigenetic changes induced by air toxics: Formaldehyde exposure alters miRNA expression profiles in human lung cells. Environ Health Perspect. 2011; 119(4):494–500. PMid: 21147603 PMCid: PMC3080931. https://doi.org/10.1289/ehp.1002614.
53. Yu G, Chen Q, Liu X, Guo C, Du H, Sun Z. Formaldehyde induces bone marrow toxicity in mice by inhibiting peroxiredoxin 2 expression.Mol Med Rep. 2014; 10(4):1915–20. PMid: 25109304. https://doi.org/10.3892/mmr.2014.2473.
54. Klaassen, CD. [ed]. Basic science of poison. Casarett and Doull's Toxicology, 6th ed. New York, NY, McGraw-Hill, 2001. p. 1005.
55. Poirier M, Fournier M, Brousseau P, Morin A. Effects of volatile aromatics, aldehydes, and phenols in tobacco smoke on viability and proliferation of mouse lymphocytes. J Toxicol Environ Health A. 2002; 65(19):1437–51. PMid: 12396875. https://doi.org/10.1080/00984100290071342 .
56. Weng M, Lee H, Park S, Hu Y, Wang H, Chen L, *et al*. Aldehydes are the predominant forces inducing DNA damage and inhibiting DNA repair in tobacco smoke carcinogenesis. PNAS. 2018; 115(27):E6152–E616. PMid: 29915082 PMCid: PMC6142211. https://doi.org/10.1073/pnas.1804869115.
57. Kumar A, Bhartiya D, Kaur J, Kumari S, Singh H, Saraf D, Sinha Narain SD, Mehrotra R. Regulation of toxic contents of Smokeless Tobacco products. Indian J Med Res. 2018; 148(1): 14–24. PMid: 30264750 PMCid: PMC6172907. https://doi.org/10.4103/ijmr.IJMR_2025_17.
58. Agency for Toxic Substances and Disease Registry [ATSDR]. Toxicological profile for Arsenic. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. 2007. 2020. https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=22&tid=3.
59. Agency for Toxic Substances and Disease Registry [ATSDR]. Toxicological profile for Beryllium. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. 2002. 2020. https://www.atsdr.cdc.gov/phs/phs.asp?id=339&tid=33.
60. Agency for Toxic Substances and Disease Registry [ATSDR]. Toxicological profile for Cadmium. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. 2012. 2020. https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=48&tid=15.
61. Agency for Toxic Substances and Disease Registry [ATSDR]. Toxicological profile for Chromium. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. 2012. 2020. https://www.atsdr.cdc.gov/substances/toxsubstance.asp?toxid=17.
62. Agency for Toxic Substances and Disease Registry [ATSDR]. Toxicological profile for Nickel. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. 2005. 2020. https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=245&tid=44.
63. Agency for Toxic Substances and Disease Registry [ATSDR]. Toxicological profile for Lead. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. 2007. 2020. https://www.atsdr.cdc.gov/PHS/PHS.asp?id=92&tid=22.
64. Agency for Toxic Substances and Disease Registry [ATSDR]. Toxicological profile for Mercury. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. 1999. 2020. https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=115&tid=24.
65. Agency for Toxic Substances and Disease Registry [ATSDR]. Toxicological profile for Selenium. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. 2003. 2020. https://www.atsdr.cdc.gov/PHS/PHS.asp?id=151&tid=28.
66. Pappas RS. Toxic elements in tobacco and in cigarette smoke: Inflammation and sensitization. Metallomics. 2011; 3(11):1181–98. PMid: 21799956 PMCid: PMC4542087. https://doi.org/10.1039/c1mt00066g.
67. Arain SS, Kazi TG, Afridi HI, Talpur FN, Kazi AG, Brahman KD. Correlation of arsenic levels in Smokeless Tobacco products and biological samples of oral cancer patients and control consumers. Biol Trace Elem Res. 2015; 168(2):287– 95. PMid: 25975948. https://doi.org/10.1007/s12011-015-0355-y.
68. WHO [World Health Organisation]. Evaluation of certain contaminants in food. 2011. 2020. https://apps.who.int/iris/bitstream/handle/10665/44514/WHO_TRS_959_eng.pdf?sequence=1.
69. National Center for Biotechnology Information. PubChem Database. Arsenic, CID=5359596. 2020. https://pubchem.ncbi.nlm.nih.gov/compound/Arsenic.
70. Byrd DM, Roegner ML, Griffiths JC, Lamm S, Grumski KS, Wilson R, *et al*. Carcinogenic risks of inorganic arsenic in perspective. 1996; 68(6):484–94. PMid: 8891790. https://doi.org/10.1007/s004200050098.

71. Abernathy CO, Thomas DJ, Calderon RL. Health effects and risk assessment of Arsenic. Nutr J. 2003; 133(5):1536S–8S. PMid: 12730460. https://doi.org/10.1093/jn/133.5.1536S.
72. Lindberg AL, Sohel N, Rahman M, Persson LA, Vahter M. Impact of smoking and chewing tobacco on arsenic-induced skin lesions. Environ Health Perspect. 2010; 118:533–8. PMid: 20064784 PMCid: PMC2854731. https://doi.org/10.1289/ehp.0900728.
73. National Center for Biotechnology Information. PubChem Database. Beryllium, CID=5460467. 2020. https://pubchem.ncbi.nlm.nih.gov/compound/Beryllium.
74. Bruce MR, Odin M. Concise international chemical assessment Document 32. World Helath Organisation, Geneva. 2001. 2020. https://www.who.int/ipcs/publications/cicad/en/cicad32.pdf?ua=1.
75. National Center for Biotechnology Information. PubChem Database. Cadmium, CID=23973. 2020. https://pubchem.ncbi.nlm.nih.gov/compound/Cadmium.
76. García-Rico L, Leyva-Perez J, Jara-Marini ME. Content and daily intake of copper, zinc, lead, cadmium and mercury from dietary supplements in Mexico. Food Chem Toxicol. 2007; 45:1599–605. PMid: 17418927. https://doi.org/10.1016/j.fct.2007.02.027.
77. Prabhakar V, Jayakrishnan G, Nair SV, Ranganathan B. Determination of trace metals, moisture, pH and assessment of potential toxicity of selected Smokeless Tobacco products. Indian J Pharm Sci. 2013; 75(3):262–9. PMid: 24082341 PMCid: PMC3783743. https://doi.org/10.4103/0250-474X.117398.
78. WHO [World Health Organization]. The Scientific Basis of Tobacco Product Regulation: Fourth Report of a WHO Study group. 2013. 2020. https://www.who.int/tobacco/publications/prod_regulation/trs_967/en/.
79. Ganguly K, Levanen B, Palmberg L, Akesson A, Linden A. Cadmium in tobacco smokers: A neglected link to lung disease? Eur Respir Rev. 2018; 27(147):pii.170122. PMid: 29592863. https://doi.org/10.1183/16000617.0122-2017.
80. National Center for Biotechnology Information. PubChem Database. Chromium, CID=23976. https://pubchem.ncbi.nlm.nih.gov/compound/Chromium.
81. Akhtar A, Afridi HI, Kazi TG. *et al*. Chromium exposure in the adult population, consuming different types of Smokeless Tobacco products in Pakistan. Biol Trace Elem Res. 2017; 175:312–21. PMid: 27422637. https://doi.org/10.1007/s12011-016-0801-5 .
82. Sogor C, Gaspar A, Posta J. Flame atomic absorption spectrometric determination of total chromium and Cr(VI) in cigarette ash and smoke using flow injection/hydraulic high-pressure sample introduction. Microchem J. 1998; 8:251–5. https://doi.org/10.1006/mchj.1997.1552.
83. National Center for Biotechnology Information. PubChem Database. Mercury, CID=23931. 2020. https://pubchem.ncbi.nlm.nih.gov/compound/Mercury.
84. Dhaware D, Deshpande A, Khandekar RN, Chowgule R. Determination of toxic metals in Indian Smokeless Tobacco products. Scientific World Journal. 2009; 9:1140. PMid: 19838600 PMCid: PMC5823206. https://doi.org/10.1100/tsw.2009.132.
85. Jarup, L. Hazards of heavy metal contamination. Br Med Bull. 2003; 68: 167–82. PMid: 14757716. https://doi.org/10.1093/bmb/ldg032.
86. National Center for Biotechnology Information. PubChem Database. Nickel, CID=935. 2020. https://pubchem.ncbi.nlm.nih.gov/compound/Nickel.
87. Linneberg A, Nielsen NH, Menne T, Madsen F, Jorgensen T. Smoking might be a risk factor for contact allergy. J Allergy Clin Immunol. 2003; 111:980–4. PMid: 12743561. https://doi.org/10.1067/mai.2003.1394.
88. National Center for Biotechnology Information. PubChem Database. Lead, CID=5352425. 2020. https://pubchem.ncbi.nlm.nih.gov/compound/Lead.
89. Kauffman JF, Westenberger BJ, Robertson JD, Guthrie J, Jacobs A, Cummins SK. Lead in pharmaceutical products and dietary supplements. Regul Toxicol Pharmacol. 2007; 48:128–34. PMid: 17467129. https://doi.org/10.1016/j.yrtph.2007.03.001.
90. Mrugesh T, Dipa L, Manishika G. Effect of lead on human erythrocytes: An in vitro study. Acta Pol Pharm. 2011; 66:653–6.
91. Jhamtani RC, Shukla S, Dahiya MS, Agarwal R. Evaluation of acute effect of lead at sub-lethal concentrations at Zebrafish. Res J Environ Toxicol. 2017; 11:97–103. https://doi.org/10.3923/rjet.2017.97.103.
92. National Center for Biotechnology Information. PubChem Database. Selenium, CID=6326970. 2020. https://pubchem.ncbi.nlm.nih.gov/compound/Selenium.
93. Hechc SS. Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines. Chem Res Toxicol. 1998; 11:559. PMid: 9625726. https://doi.org/10.1021/tx980005y .
94. Stepanov I, Hecht SS, Ramakrishnan S, Gupta, PC. Tobacco-specific nitrosamines in Smokeless Tobacco products marketed in India. Int J Cancer. 2005; 116(1):16–9. PMid: 15756678. https://doi.org/10.1002/ijc.20966.
95. Pickworth WB. Smokeless Tobacco products: Characteristics, usage, health effects and regulatory implications. Elsevier. 2020.

96. National Cancer Institute and Centers for Disease Control and Prevention. Smokeless Tobacco and Public Health: A Global Perspective. Bethesda, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Institutes of Health, National Cancer Institute. NIH Publication No. 14-7983; 2014. 2020. https://cancercontrol.cancer.gov/brp/tcrb/global-perspective/SmokelessTobaccoAndPublicHealth.pdf.
97. Cogliano V, Straif K, Baan R, Grosse Y, Secretan B, El Ghissassi F. Carcinogenicity of human papillomaviruses. Lancet Oncol. 2004; 5(12:708. https://doi.org/10.1016/s1470-2045(05)70086-3.
98. EPA [Environmental Protection Agency]. N-Nitrosomorpholine, 2000. 2020. https://www.epa.gov/sites/production/files/2016-09/documents/n-nitrosomorpholine.pdf.
99. HSFS [Hazardous Substance Fact Sheet]. New Jersey Department of health and Senior Services. 2006. 2020. https://nj.gov/health/eoh/rtkweb/documents/fs/1412.pd/f.
100. National Center for Biotechnology Information. PubChem Database. N-Nitrososarcosine, CID=25811. https://pubchem.ncbi.nlm.nih.gov/compound/N-Nitrososarcosine.
101. National Center for Biotechnology Information. PubChem Database. N-Nitrosonornicotine, CID=12613538. 2020. https://pubchem.ncbi.nlm.nih.gov/compound/3-_2S_-1-nitrosopyrrolidin-2-yl_pyridine.
102. National Center for Biotechnology Information. PubChem Database. N-Nitrosodiethanolamine, CID=14223. 2020. https://pubchem.ncbi.nlm.nih.gov/compound/N-Nitrosodiethanolamine.
103. National Center for Biotechnology Information. PubChem Database. N-Nitrosomorpholine, CID=6046. 2020. https://pubchem.ncbi.nlm.nih.gov/compound/N-Nitrosomorpholine.
104. National Center for Biotechnology Information. PubChem Database. N-Nitrosopiperidine, CID=7526. 2020. https://pubchem.ncbi.nlm.nih.gov/compound/N-Nitrosopiperidine.
105. National Center for Biotechnology Information. PubChem Database. N'-Nitrosoanabasine, CID=14335. 2020. https://pubchem.ncbi.nlm.nih.gov/compound/N_-Nitrosoanabasine.
106. National Center for Biotechnology Information. PubChem Database. N-Nitrosoanatabine, CID=51291. 2020. https://pubchem.ncbi.nlm.nih.gov/compound/N-Nitrosoanatabine.
107. Patten CJ, Smith TJ, Friesen MJ, Tynes RE, Yang C S, Murphy SE. Evidence for cytochrome P450 2A6 and 3A4 as major catalysts for N'-nitrosonornicotine alpha-hydroxylation by human liver microsomes. Carcinogenesis. 1997; 18(8):1623–30. PMid: 9276639. https://doi.org/10.1093/carcin/18.8.1623.
108. Peterson LA. Formation, repair, and genotoxic properties of bulky DNA adducts formed from tobacco-specific nitrosamines. J Nucleic Acids. 2010: 284935. PMid: 20871819 PMCid: PMC2943119. https://doi.org/10.4061/2010/284935.
109. Sturla SJ, Scott J, Lao Y, Hecht SS, Villalta PW. Mass spectrometric analysis of relative levels of pyridyloxobutylation adducts formed in the reaction of DNA with a chemically activated form of the tobacco-specific carcinogen 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone. Chem Res Toxicol. 2005; 18(6):1048–55. PMid: 15962940. https://doi.org/10.1021/tx050028u
110. Wong HL, Murphy SE, Hecht SS. Cytochrome P450 2A-catalyzed metabolic activation of structurally similar carcinogenic nitrosamines: N '-Nitrosonornicotine enantiomers, N-Nitrosopiperidine and N-nitrosopyrrolidine. Chem Res Toxicol. 2005; 18(1):61–9. PMid: 15651850. https://doi.org/10.1021/tx0497696 .
111. Yalcin E, Monte SJ. Tobacco nitrosamines as culprits in disease: Mechanisms reviewed. Physiol Biochem. 2016; 72(1):107–20. PMid: 26767836 PMCid: PMC4868960. https://doi.org/10.1007/s13105-016-0465-9.
112. Vu AT, Taylor KM, Holman MR, Ding YS, Hearn B, Watson CH. Polycyclic aromatic hydrocarbons in the mainstream smoke of popular U.S. cigarettes. Chem Res Toxicol. 2015; 28(8):1616–26. PMid: 26158771 PMCid: PMC4540633. https://doi.org/10.1021/acs.chemrestox.5b00190 .
113. Agency for Toxic Substances and Disease Registry [ATSDR] U.S. Department of Health and Human Services, Public Health Service; Atlanta, GA: Toxicological Profile for Polycyclic Aromatic Hydrocarbons [PAHs]. 1995. https://www.atsdr.cdc.gov/PHS/PHS.asp?id=120&tid=25.
114. Gerde P, Scholander P. A mathematical model of the penetration of polycyclic aromatic hydrocarbons through the bronchial lining layer. Environ Res. 1987; 44(2):321–34. https://doi.org/10.1016/S0013-9351[87]80241-4 .
115. Harvey RG. Environmental chemistry of PAHs. In PAHs and related compounds. Springer, Berlin, Heidelberg; 1998: 1–54. https://doi.org/10.1007/978-3-540-49697-7_1.
116. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. IARC Monographs on the evaluation of carcinogenic risks to humans. 2010; 92:1.

117. Moorthy B, Chu C, Danielle JC. Polycyclic aromatic hydrocarbons: From metabolism to lung cancer. Toxicol Sci. 2015; 145(1):5–15. PMid: 25911656 PMCid: PMC4408964. https://doi.org/10.1093/toxsci/kfv040.
118. Orisakwe OE, Igweze ZN, Okolo KO, Udowelle NA. Human health hazards of poly aromatic hydrocarbons in Nigerian Smokeless Tobacco. Toxicol Rep. 2015; 2:1019–23. PMid: 28962443 PMCid: PMC5598458. https://doi.org/10.1016/j.toxrep.2015.07.011