Review Article

GENOTOXIC EFFECTS OF CIGARETTE AND MARIJUANA SMOKING

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EFFECTS OF TOBACCO AND MARIJUANA USE

There is strong epidemiological evidence that tobacco and marijuana cause cancer in humans. This carcinogenicity has been confirmed by experiments in animal models (1,2).

Cigarette smoking has been associated with cancer of the lungs, larynx, oral cavity, esophagus, pancreas, and bladder. Tobacco chewing has also been associated with increased incidence of cancer of the oral cavity and esophagus (3). Cigarette smoke condensate was reported to produce skin cancer in mice in 1953 (4). Nitroso compounds in cigarette smoke were found to be teratogenic in rats when administered by the respiratory route (5).

An increase in congenital defects was found among children of smoking mothers, which was partly credited to a substantial increase in the carboxyhemoglobin concentration of fetal blood (6,7). In one study, women who smoked twenty or more cigarettes daily had a relative risk of 1.6 for congenital malformation in the offspring compared to nonsmokers. The risk was greater for chromosomal abnormalities of the digestive system, heart valves, skin, and neural tube (8). There was also increased occurrence of spontaneous abortion.

In a study of 5200 pregnancies, there was a significant increase in perinatal mortality when the fathers smoked more than ten cigarettes per day. Heavier paternal smoking habits increased the frequency of stillbirths and of major congenital malformation (9). This may be due to smoke inhalation by the mother or to sperm cells made defective by the excessive smoking.

Marijuana consumption has been associated with abnormalities of mitosis and impairment of contact inhibition. Clinical and laboratory experiments showed that marijuana smoking caused bronchial irritations, lung tissue abnormalities characteristic of the early stages of cancer, and other more pronounced abnormalities (10,11). Tar from marijuana cigarettes elicited neoplastic changes in mouse skin as well as squamous metaplasia and acanthosis in sebaceous glands (12).

Both tobacco and marijuana smoke were observed to increase chromosome breakage in cultured hamster lung cells (11).

COMPONENTS OF TOBACCO AND MARIJUANA LEAVES AND SMOKE CONDENSATE

Mutagenic properties of tobacco leaves. Tobacco leaf extracts were studied to locate the leaf constituents responsible for biologic activity. The flue-cured tobacco was distilled by Soxhlet with a series of organic solvents of increasing polarity, ranging from hexane, chloroform, acetone, to methanol. The residue was blended and finally extracted with water (13). Free nicotine was extracted by chloroform and bound nicotine by later solvents. The aqueous extract contained polyphenolic pigments.

It has been suggested that the age of tobacco leaves can influence mutagenic potency. Smoke condensate from old leaves was less mutagenic than those prepared from young leaves. Also, smoke condensate from tobacco with high sugar content, which is said to be low in nitrogenous components, was less mutagenic than that from tobacco with less sugar.

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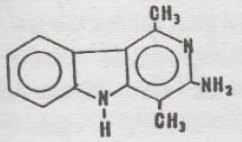
Mutagens and carcinogens in cigarette smoke condensate. Tumor-promoters reside primarily in the basic, the weakly polar, and the weakly acidic fractions of the smoke condensate (14,15,16). Mutagenicity of cigarette smoke condensates was attributed to nitrogen components: nitrates, proteins and amino acids (17,18). Some of the potential mutagens formed from proteins and amino acids during the burning of cigarettes are:



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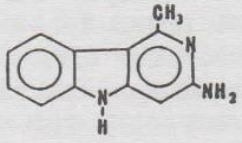
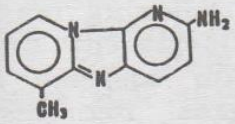
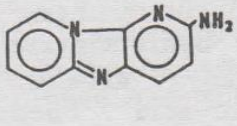


Trp p-2

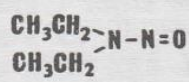


Glu p-1

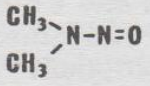


Glu p-2

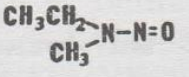
Several investigators have identified varying quantities of volatile nitrosoamines in cigarette smoke, among which are dimethylnitrosamine, diethylnitrosamine, ethylmethylnitrosamine, and di-N-propylnitrosamine (19). N-nitrosoamines are easily formed by the reaction of secondary amines with nitrite and, to a lesser extent, by the reaction of tertiary amines with nitrites.



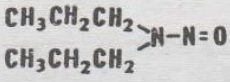
diethylnitrosamine



dimethylnitrosamine



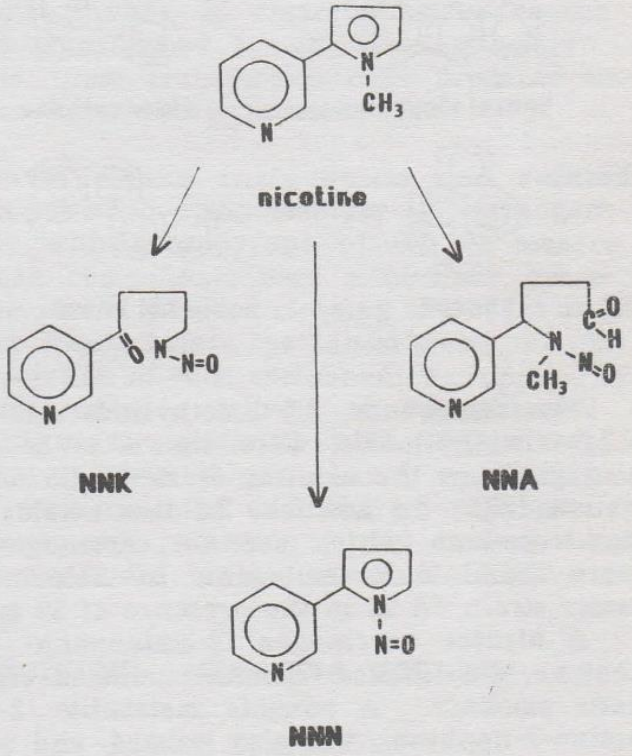
ethylmethylnitrosamine



di-N-propylnitrosamine

It has been demonstrated repeatedly that a number of N-nitroso compounds are carcinogenic, mutagenic, or both, in a wide variety of experimental animals. Although there is no direct evidence that these compounds are human carcinogens, they should be regarded as potential hazards (20).

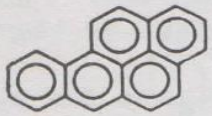
The tobacco alkaloids nicotine and nornicotine are possible precursors of N-nitrosonornicotine or NNN and (4-N-methyl-N-nitrosoamino-1-(3-pyridyl-1-butanone) or NNK, increasing the already high levels of NNN and NNK in unburned tobacco and in mainstream and sidestream tobacco smoke (21,22,23,24). NNN has been identified in unburned commercial tobacco products in amounts ranging from 0.3 to 90 ppm. The structural relationships of nicotine, NNN, and NNK are as shown:



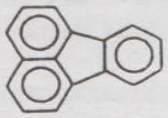
NNN, which is nonvolatile and separable into anti and syn conformers, induced lung adenomas in mice, esophageal and nasal tumors in rats and tracheal tumors in hamsters (21,25). Nasal cavity tumors were found to be in 92% of the male rats and 75% of the females. Only one liver tumor and no lung tumor was observed in NNN-treated rats. NNK induced nasal cavity tumors in 83% of the males and 13% of the females, liver tumors in 83% of the males and 100% of the females, and lung tumors in 67% of both

males and females. NNK was found to be a more powerful carcinogen than NNN in F344 rats.

The largest known group of chemical carcinogens in cigarette smoke, credited with the major carcinogenic activity of smoke condensate, are the polynuclear aromatic hydrocarbons (PAH). Some 150 PAH in smoke were quantitated and identified as to parent ring structures and type of alkyl substituents (26). These include benzo(a)pyrene, benzofluoranthene, fluoranthene, pyrene, chrysene, perylene, anthracene, fluorene, and benz(a)anthracene.

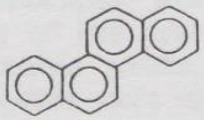


benzo(a)pyrene



fluoranthene

pyrene



chrysene

Two compounds, 2,3-dimethylindole and 2,3,5-trimethylindole, were shown to be mutagenic in the absence of metabolic activation (27). In addition, 2,6-diaminotoluene and coronene, which are not carcinogenic were found to be mutagenic to *Salmonella* tester strain TA 98 in the presence of S9 mix.

A bladder carcinogen, 2-aminonaphthalene, was isolated from the urine of cigarette smokers. A possible metabolite, 2-amino-7-naphthol, was also isolated, and was shown to be mutagenic. These suggest that aromatic amines may also be responsible for some of the mutagenicity of cigarette smokers (28).

Several other compounds present in cigarette smoke such as benzo(a)pyrene, urethane, anthracene, nitrosamine, and hydrazines, have been shown to be transplacental carcinogens in laboratory animals (29).

Cigarette smoke condensate promoted the mutagenic effects of benzo(a)pyrene and 2-acetylaminofluorene (30,31). This was shown for benzo(a)pyrene by the transformation of hamster embryo cells.

Cocarcinogens and tumor promoters in cigarette smoke condensate. It is clear that the complete carcinogenic effect of smoke condensate cannot be due solely to identified carcinogens. Studies have shown a tumor promoting effect of the weakly acidic fraction of the smoke and a cocarcinogenic effect of catechol as well as for various neutral smoke constituents (16).

In another study, a series of 21 tobacco smoke components and related compounds were tested for cocarcinogenic activity on mouse skin (32). The compounds were applied to the shaved backs of the mice three times weekly with 5 µg benzo(a)pyrene. The following compounds remarkably enhanced the carcinogenicity of benzo(a)pyrene: catechol, pyrogallol, decane, undecane, pyrene, benzo(e)pyrene, and fluoranthene. The following inhibited the benzo(a)pyrene carcinogenicity completely: esculin, quercitin, squalene, and oleic acid. Phenol, eugenol, resorcinol, hydroquinone, hexadecane, and limonene were partial inhibitors. Six of the 21 compounds were also tested as tumor promoters in two-stage carcinogenesis. Co-carcinogens pyrogallol and catechol did not show tumor-promoting activity. Decane, tetradecane, anthralin, and phorbol myristyl acetate showed both types of activity.

Catechol and pyrogallol are two of the most potent cocarcinogens. Catechol is the most abundant phenol in cigarette smoke condensate although it is not found in tobacco leaf. The yield of catechol is 0.4 to 0.5 mg per cigarette from 85 mm nonfilter cigarettes and 0.2 to 0.3 mg from filter cigarettes (33). The other compounds such as olefinic and aliphatic hydrocarbons and long-chain fatty acids and alcohols occur in both tobacco leaf and smoke (35).

Mutagenic activities of marijuana smoke condensate. This was found to be comparable to that of tobacco smoke (35). Most of the mutagenicity resides in the basic fraction, indicating that amino acid and protein pyrolyzates may be accountable. Tumor-initiating activity is found mainly in the neutral fraction.

ASSAY SYSTEMS USED TO DETERMINE TOXICITY

Tumor promoters in tobacco leaf. In the serial extraction of tobacco leaf with solvents of increasing polarity, a total of 5 fractions were obtained (13). Promoting activity of the test fractions were determined using 50 female ICR Swiss mice (59-67 days old). The shaved dorsal area was applied with 125 µg of 7,12-dimethylbenz(a)-anthracene in 0.25 mL acetone. After three weeks, the mice were treated 5 times with 0.2 mL of each of the test solutions, the bioassay being continued for 52 weeks. The mice were examined weekly for skin tumors 1 mm or larger in diameter.

Smoke condensate. Cigarette smoke condensate transformed cell cultures, converting hamster lung fibroblasts into criss-crossed fusiform cells (36). Tumors developed in the cheek pouch of young adult hamsters after they were inoculated with these fusiform cells. It was also demonstrated that the condensate-treated cells caused tumors when injected into newborn C3H mice.

Condensates were also tested for induction of sister chromatid exchanges (SCE) in ovary cells of Chinese hamster and for mutations in *Salmonella typhimurium* The responsive components were found to be different for both tests (37).

When glycerol was added to tobacco smoke condensate in acetone solvent, the topical carcinogenicity and the ability to produce epithelial hyperplasia in mice was reduced (38). This was determined using two doses of condensate, 189 and 94.5 mg/mouse/week, combined with two concentrations of glycerol. Each mouse was clipped from the base of the tail to the nape with an electric hair clipper lubricated with liquid paraffin. Unpainted controls were clipped once a week, and equally handled. Treatment with the condensate-acetone solution was made after two weeks, when mice were 6-7 weeks old, and continued until their death or until 108 weeks of treatment had been completed. The mice were painted with each of these 2 doses of condensate and subsequently painted or not painted with equivalent volumes of solvent or solvent/glycerol. Papillomas and suspected sebaceous adenomas were recorded when they appeared to be greater than 1 mm^3 and had been present for two consecutive weekly injections.

Smoke condensate extracts. Four neutral fractions of cigarette smoke condensates were added to organ cultures of human fetal lung. All induced hyperplasia of the bronchial epithelial lining. Using the same organ culture system, it was demonstrated that an enriched hydrocarbon fraction was a potent inducer of hyperplasia and other cytological changes indicative of malignant transformation (39).

Other studies showed that a basic and an acidic fraction of cigarette smoke condensate transformed Syrian hamster embryo cells; these transformed cells produced tumors in newborn hamsters (40).

Known smoke components and related compounds. The test for cocarcinogenicity with benzopyrene of the 21 tobacco smoke components used a bioassay procedure which included clipping the backs of 50 mice 2 days before the initial treatment (32). A solution of each component was applied by micropipette in acetone or by calibrated paint brush in dimethylsulfoxide, 3 times weekly. Tumors bigger than 1 mm in diameter were counted and charted regularly. Only tumors persisting for 30 days or more were included in the cumulative totals.

CONDITIONS AFFECTING TOXICITY

Tobacco type. Mutagenic activity was greater with cigar tobacco than cigarette tobacco, and least with pipe tobacco. Aqueous extracts of flue-cured and cigarette tobacco appeared about 5 times as active as smoke condensate derived from an equal weight of tobacco (13). Bioassay results showed tumor-promoting activity for the aqueous extract in 38% of the experimental animals.

The smoke condensate from cigarette, cigar, and pipe tobacco were also investigated using *Salmonella* tester strains TA 100 and TA 98. Most of the condensates had

detectable mutagenic activity in TA 100 in the absence of S9 (liver homogenates). It was also found that TA 98 was more sensitive than TA 100 in the various condensates tested, suggesting that frameshift mutagens account for most of the mutagenicity of tobacco smoke condensates (41).

Cigarette filters. The decline in lung cancer risk which eventually becomes apparent when an individual changes from non-filter to filter cigarettes with a lower tar yield, might be due, in part, to a reduction of tumor enhancers (42). This might also relate to the selective reduction of weekly acidic components by smoke filtration.

Cigarette smoke condensate from high charcoal filter cigarette was shown to be mutagenic, indicating that such filters do not prevent the passage of certain mutagens into the lungs of the smokers (43). The condensate from low nicotine cigarettes gave the same quantitative response as the high charcoal filter cigarettes.

Tar content. The mutagenicity per mg of cigarette smoke condensate was nearly the same for low tar and high tar cigarettes (41). The specific mutagenicity of cigarette smoke condensate is therefore not dependent on the amount of tar in the entire cigarette.

Cigarette section. Mutagenicity of condensate from various sections of cigarette was found to be almost the same for the first, the middle, or last third of the cigarette. Specific mutagenicity was the same whether the condensate was from filter or from non-filter cigarettes.

Mainstream and sidestream smoke. Sidestream smoke condensate induced higher aryl hydrocarbon hydroxylase activity in pulmonary tissue than mainstream smoke condensate (44). Mainstream smoke gave no mutagenic response in the absence of S9 but high response with TA 98 plus S9. Sidestream smoke gives a low response with TA 98 with S9 which is increased with TA 98 plus S9. Sidestream smoke is mutagenic in the absence of S9 in TA 98 and TA 104, in contrast to mainstream smoke (45).

Smoking frequency. Adult men who smoked more than 10 cigarettes per day had a greater SCE frequency than those who smoked less (46). Furthermore, it was shown that the mean SCE frequency of those who smoked for over 10 years, irrespective of the number of cigarettes, was greater than those who smoked for less than 10 years.

Other factors. Smoke condensates were shown to cause frameshift mutations when activated by microsomal enzymes (47). The crude smoke condensate, reconstituted condensates, and all active fractions, required the presence of S9 mix for the detection of any mutagenic activity towards TA 1538. The nitrate-treated condensate differed significantly from the other smoke condensate in its lack of microsomal preparation for mutagenic activity towards TA 1538 and its ability to revert the base-pair substitution mutation in TA 1535. The nitrate treatment may have produced new types of mutagenic agents not found in the other condensates.

Indoor air contaminated with tobacco smoke caused significant sister chromatid exchange both in the presence and the absence of exogenous metabolizing system (48).

Marijuana smoke. Mutagenicity was also associated with nitrogen content of marijuana smoke (49). The test systems used were *Salmonella* strains TA 98, TA 100, TA 1537 and TA 1538. Marijuana was most mutagenic followed by pipe tobacco and cigarette tobacco.

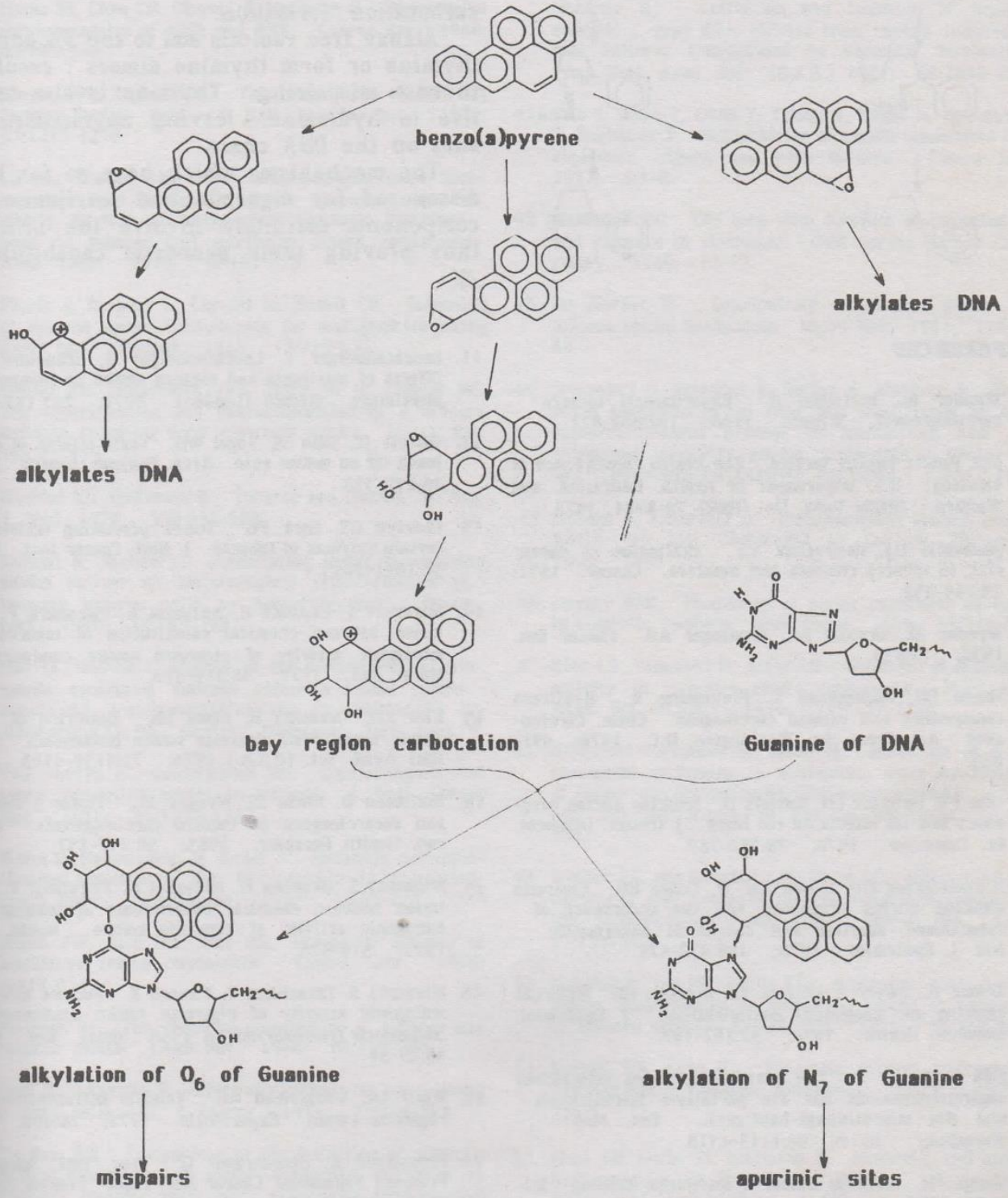
Results of comparative analysis of polynuclear aromatic hydrocarbons in marijuana and tobacco smoke indicate a considerably higher content of potential carcinogens in the former. Pyrolysis products of cannabinoids are major contributors to PAH (50).

REACTIVITY OF SOME GENOTOXIC AGENTS

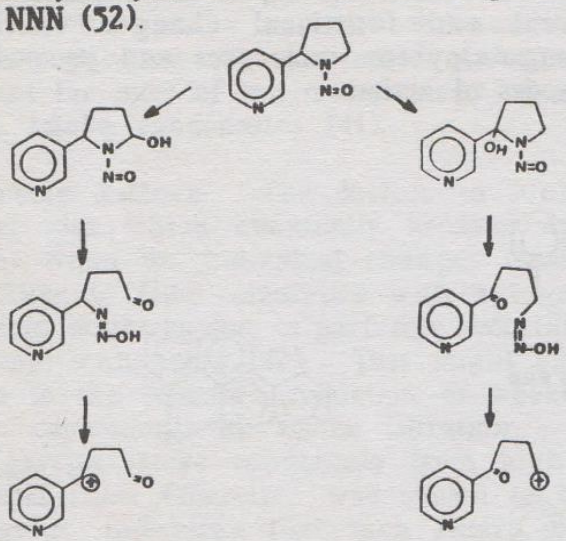
One of the enzymes involved in the metabolism of the polycyclic hydrocarbons is aryl

hydrocarbon hydroxylase. This is inducible in mouse skin after treatment with a fraction of cigarette smoke condensate containing a high concentration of PAH. A single application of tumorigenic cigarette smoke condensate subfractions causes a 5- to 8-fold increase in epidermal aryl hydrocarbon hydroxylase (51).

The following figure shows the structural and functional changes which benzo(a)pyrene undergoes and its possible modes of action.



Metabolic experiments with NNN *in vitro* and *in vivo* demonstrate conclusively that alpha-hydroxylation is a metabolic process for NNN (52).



N-nitrosoamines undergo hydroxylation to form oximes, which are converted to nitriles upon dehydration. The carbocation produced upon nitrogen release can react with DNA, alkylating O_6 of guanine , causing mispairs, or N_7 also of guanine, leaving an apurinic site. The same results are obtained with amino acid pyrolyzates like Trp p-2, after it has undergone N-hydroxylation and carbocation formation.

Alkoxy free radicals add to the 5,6 bond of thymine or form thymine dimers , resulting in base mispairing. Thymine is also sensitive to hydrazines, leaving apyrimidinic sites on the DNA chain.

The mechanisms which have so far been discovered for cigarette and marijuana components definitely involve the DNA, thus proving their genotoxic capabilities.

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