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Nitrosamines: their implication to human cancer through industrial and environmental exposure

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니토로스아민류 : 産業과 環境폭로에 의한 癌생성

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I. Introduction

The toxicity and carcinogenicity of nitrosamines is a subject of concern because of wide human exposure to this group of compounds. Nitrosamines have been found in the environment, processed foods (smoked herring, smoked sausage, ham and bacon), vegetables, tobacco, cigarette smoke, alcoholic beverages, air and water (1-5). In addition to environmental sources, nitrosamines can be derived from several industries, such as leather tanning, rubber and rocket fuel industries (6). Other sources include cosmetics, pesticides and pharmaceuticals. Also, there is the evidence that nitrate and nitrite salts are both formed and destroyed endogenously by microorganisms or metabolic action and produce nitrosamines (7).

Nitrosamines, in which the nitroso groups (-N=0) are attached to nitrogen atoms of chemicals, first made toxicologic interest in 1954, when Barnes and Magee reported on the hepatotoxicity of dimethylnitrosamines (8). In 1956, they demonstrated the production of primary malignant hepatic tumor in rats by feeding 50 ppm of dimethylnitrosamine for 5 to 10 months (9). Recently, much research has proven that these compounds can cause tumors in various organs or tissues of most animal species tested and suggested potential carcinogens for humans (10-13). Other toxicities which have been found for these compounds include cytotoxicity, mutagenicity and teratogenicity in animals, According to epidemiological studies, nitrosamines were reported to be causative for stomach and esophageal cancer (14-16).

Based on animal and human data, IARC (International Agency for Research on Cancer) Working Group concluded that N-nitrosodimethylamine were probably carcinogenic to humans.

II. The Chemistry of Nitrate, Nitrite and Nitrosamines (R_1R_2 NNO)

Nitrates are ubiquitous in our environment because they can be formed either naturally or anthropogenically. In water, animal wastes,

decaying organic matters, nitrogen fertilizers and soil high in nitrogen-fixing bacteria may be sources of nitrate contamination. Nitrates also come from fuel combustion in the air and they are circulating in the environment among water, air and soil through the nitrogen cycle, where nitrification from ammonia to nitrite and nitrate, denitrification from nitrate to molecular nitrogen and nitrogen fixation process from molecular nitrogen to ammonia are occuring (17, 18). Although some of nitrate compounds, for example, the peroxyacyl nitrates (PANs), are eye irritants and may be toxic at ambient levels, nitrate per se is relatively nontoxic to human. The health hazards that are associated with nitrates result chiefly from the reduction of nitrates to nitrites because nitrites, more reactive and stronger nucleophiles than the nitrite ion, readily react with amines to form N -nitroso compounds, such as nitrosamines and nitrosoamides. Generally, the reduction of nitrate ion to either nitrite ion (NO_2^-) or nitrogen dioxide occurs in acidic condition. Therefore, the ubiquitous presence of nitrates can contribute to the formation of nitrosamines around us.

III. Formation and Inhibition of Nitrosamines

The process for the formation of nitrosamines is called nitrosation that amino substrates are nitrosated by electrophilic nitrosating agents derived from nitrous acid, dinitrogen trioxide, dinitrogen tetroxide and nitric acid (19). Nitrosation usually occurs from the reaction of compounds that can generate a nitrosonium ion is obtained in strong acids or available from nitrosating agents.

The first example of nitrosamine formation is the nitrosation of amines by aqeous nitrous acid (a). Although secondary amines are better substrates for nitrosation, primary, tertiary and quarternary amines can also be utilized as substrates to form nitrosamines (20). In this reaction, but only nitrosating agents (Y-NO), formed from the nucleophilic catalyst (Y^-:NO_2^-, CL^-, SCN^-) and protonated nitrous acid (H_2O^+-NO), react with amino substrates. This reaction depends on pH (<5), basicity of the amino substrates, concentration of nitrite ion and amino substrate and the presence of catalysts.

(a) Aqueous nitrous acid and amines at the optimal pH=3.4

$NO_2^- + H_3O^+ \longrightarrow HNO_2 + H_2O$

$HNO_2 + H_3O^+ \longrightarrow H_2O\text{-}NO \xrightarrow{Y^-} Y\text{-}NO + H_2O$

$R_2NH + Y\text{-}NO \longrightarrow R_2NNO + HY$

$Rate = K_1(R_2NH)\,(Nitrite)^2$ (R=aliphatic or aromatic hydrocarbons)

(b) Acidified nitrous acid and weakly basic amines

$R_2NH + H_2ONO^+ \xrightarrow{slow} R_2NNO + H_3O^+$

(c) Nitrosation by Nitrogen Oxides (N_2O_3, N_2O_4 etc)

$R_2NH + N_2O_3(N_2O_4) \xrightarrow{fast} R_2NNO + HNO_2\,(HNO_3)$

(d) Nitrosation by Microbial Activity

$R_2NH + Microorganisms + HNO_3 \longrightarrow R_2NNO + H_2O$

(e) Others: transnitrosation and nitrosation by nitrate and nitrite salts

(f) Inhibition: by Ascorbic acid, Vitamin E and Other Antioxidants

The nitrosation by acidified nitrous acid is quite slow at pH>3, but it becomes faster with increasing acidity (b). This reaction depends on

the structure of amines (21). Choi recently established a mathematical model to create nitrate, nitrite and N-nitrosamine exposure (22). In addition to reactions (a) and (b), nitrosation occurs by nitrogen oxides generated from the chemical and microbial reduction of nitrate and nitrite salts and the combustion (c). Nitrogen dioxide and nitro oxide can nitrosate the amines, but nitric oxide requires either oxidation to nitrogen dioxide or certain catalysis, such as metal salts and iodine. No acidic conditions are required, But this reaction rate is faster than (a) and (b). Particularly, this reaction can occur in the gas phase, in lipophilicity and in neutral and alkaline aqueous solutions. In the atmosphere, nitrogen oxide alone converts secondary amines to nitrosamines (23). In organic solvents, gaseous dinitrogen trioxide reacts with amines to give high yields of nitrosamines, whereas gaseous dinitrogen tetroxide yields a mixture of N-nitroso (=NNO) and N-nitro compounds (=NNO_2).

The reduction of nitrate to nitrite by bacterial activity is an important route of nitrosamine formation (d). About 25% of ingested nitrate is recirculated into the saliva and approximately 20% of nitrate in the saliva is reduced to nitrite. Reduction of nitrate by oral bacteria, such as the general of Staphylococcus, Veillonelus, Corynebacterium and Fusobacterium, accounts for most of the nitrite that is present in the stomach where nitrosation reactions mostly occur. However, there is considerable variation in the amounts and kinds of oral bacteria in healthy individuals (24). These bacteria possess nitrate reductase enzyme so that they can convert nitrate to nitrite and finally to form nitrosamines. The optimum pH is 6.0 to 6.4 for the conversion and the enzymatic activity is often inducible. The enzyme is reported to be also present in fungi, algae and plants. Because of the pH dependency of nitrate reduction in the saliva, this reduction process can be inhibited or enhanced. Recently, the technique for estimating the extent of in vivo nitrosamine formation in human was developed by assessing urine samples (25). Hill *et al.*, (26) suggested that individuals might be at high risk of gastric cancer with the condition that favoured a profuse gastric flora due to elevated pH and that the bacteria could catalyze the formation of nitrosamines from ingested secondary amines/amides and nitrate/nitrite. Contrary to expectation, higher salivery nitrate and nitrite levels were found in the population at low rather than at high risk for gastric cancer (27). Additionally, the bacteria-mediated catalysis of nitrosation also occurs exogenously from soil or sewage (28).

Other ways of nitrosation include transnitrosation and nitrosation by nitrite salts. First, transnitrosation is the nitrosation by organic nitroso is the nitro compounds. Certain nitroso and nitro compounds can act as nitrosating agents through the transfer of their nitroso group to amines. Transnitrosation occurs at pH less than 3 and is catalyzed by nucleophilic anions, such as thiocyanate and iodine (29). The compounds for transnitrosation are alicyclic nitrosamines (nitrosoproline, nitropipecolic acid), piperazines, morpholines and alkylpiperidines. This transnitrosation occurs because N-nitroso and N-nitro compounds liberate either nitrous acid on acid treatment or nitric oxide and nitrogen dioxide during heating and thus serve as nitrosating agents. Second, nitrosamines can be formed by nitrate and nitrite salts. However, nitrate salts require reductive condition to have either nitrite ion or

nitrogen dioxide and nitric oxide intermediates and nitrite salts need the presense of ferrocyanideion at pH 11 for the production of nitrosamines (30).

As descibed above, the formation of nitrosamines is diverse and highly productive when the source of amines and nitrate or nitrite ion is in the favorable conditions. This may explain why nitrosamines are everywhere in the environment and in the industry.

In addition to nitrosation, inhibition can also occur by inhibitors. These inhibitors include ascorbic acid, α-tocopherol, sodium bisulfite, thiols, certain phenols, gallic acid and other antioxidants. In general, these inhibitors prevent nitrosating agents from forming nitrosamine by reducing them to either nitrogen or nitric oxide (31). For example, ascorbic acid blocks the formation of nitrosamines by the reduction of nitrosating agent and is effective at pH 1 to 4 in the absence of oxygen when its concentration is equal to or greater than that of nitrite. This reaction is more effective in hydrophilic media because ascorbic acid is water soluble contrary to vitamine E which is lipophilic.

IV. Sources of Nitrosamines

1. Industrial Sources

Nitrogen oxides and amines react very efficiently to form nitrosamines. This reaction is particularly responsible for the nitrosamines in the industry and in the air. In the industrial areas, workers are highly exposed to volatile nitrosamines, primarily by inhalation from various manufacturing sources (Table 1).

Table 1. Nitrosamines in the industrial sources.

Industry	Nitrosamines	Exposure Level
Rubber	NDPhA	0.2-47 (ug/m^3)
	NDMA	0.05-0.5 (ug/m^3)
	NMOR	0.5-29 (ug/m^3)
Leather	NDMA	440 ug/day/person
Tanning	NMOR	20 ug/day/person
Rocket Fuel	NDMA	1-36 (ug/m^3)
Pesticudes		
trifluralin	MADP	—
2, 3, 6-dichloro benzoic acid	NDMA	187-640 mg/l
Cosmetics		
soap or shampoo	DENA	—
others	NBMA	—
	NDOMA	
	NMSA	

* NDPhA : Nitrosodiphenylamine,
NMOR : Nitrosomorpholine,
DENA : Diethylnitrosamine,
NMSA : Nitrosomethylstearylamine,
NDMA : Dimethylnitrosamine
NDPA : Nitrosodipropylamine,
NBMA : Nitrosobenzylmethylamine
NDDMA : Nitrosododesylmethylamine
** Data sources: (2), (32)

1) Rubber Industry: The rubber industry uses a variety of organic accelerators, retardants and antioxidants for the rubber production. Among these compounds, nitroso diphenylamine (NDPhA), for example, has been extensively used as a vulcanization retarder. The carcinogenicity of this compound is not likely, but NDPhA can act as a transnitrosating agent and may contribute to the formation of other carcinogenic nitrosamines. Other N-nitroso compounds for rubber industry include nitrosofurfuramide. N-methyl-N-4-dinitrosoaniline and so on. Because of the use of N-nitroso compounds and their contribution to the nitrosation of other amines in the industry, many nitrosamines are detected with a certain variation. The air level of NDPhA was found in the range of 0.2 to 47 ug/m^3 and that of nitrosomorpholine (NMOR) was also detected at the high level ranging from 0.5

to 29 ug/m^3 (32). Additionally, dimethylnitrosamine (NDMA) was detected in the air of rubber factories at low levels (0.05 to 0.5 ug/m^3). There is an occupational exposure difference between rubber chemical production and rubber (tire curing). The maximal daily exposure to NMOR is 250 ug in rubber tire curing, but in the rubber chemical production the daily exposure to NDphA is maximally 430 ug (6).

2) **Leather Tanning:** In leather tanning industry, NDMA was found at high level. For depilatory solution, dimethylamine sulfate (DMAS) is used and is highly associated with the presence of NDMA. Presumably. dimethylamine formed from the DMAS is needed to produce NDMA. However, oxide of nitrogen formed by the combustion of fossil in gas-powered forklift trucks or in open gas heaters may cause the formation of NDMA in the air of workplace. Maximum daily exposure to NDMA is 440 ug/person and 20 ug to NMOR.

3) **Rocket Fuel Factory:** NDMA was found in rocket fuel factory because NDMA was used for the manufacture of rocket fuel-dimethylhydrazine. In Baltimore, the level of NDMA was detected in the factory site and particulary in the residential area adjacent to the factory ranging from 1 ug/m^3 to 36 ug/m^3 (2).

4) **Pesticides**: Because many pesticides are certain types of amines, substantial nitrosamine impurities occur in the formulations of pesticides after nitrosation by nitrites or other nitrosating agents. For example, nitrosodipropylamine (NDPA) was contaminated in trifluralin herbicide when nitration with nitric and sulfuric acids was followed by the addition of dipropylamine during synthesis (33). In amine salt pesticides, some dimethylamine salt formulations of 2, 3, 6-dichlorobenzoic acid contain 187 to 640 mg NDMA/liter. The use of sodium nitrite as a rust inhibitor can contribute to the nitrosation of amines in the pesticide prodution, but nitrosation may also occur through the interaction with nitrate fertilizers after the application of pesticides.

5) **Cosmetics:** Cosmetics, such as soaps and shampoos, are contaminated with diethylnitrosamines (DENA). This contamination can be derived from the reaction between the amine source of triethanolamine, which is present in most cosmetic formulations and the nitrosating agent of bronopol, which is used for the bacteriocide (34). According to the use of various amines, other types of nitrosamines, such as nitrosobenzylmethylamine (NBMA), nitrosomethylstearylamine (NMSA) and nitrosododecylmethylamine (NDOMA), can be formed during cosmetic production.

6) **Pharmaceuticals:** Many drugs are susceptible to nitrosation and may be contaminated by nitrosamines similarly to pesticides. In the Federal Republic of Germany, it was reported that 68 commercial formulations of the antipyretic analgesic drug aminopyrine contained NDMA ranging from 1-370 ug/kg (35). This aminopyrine, a pyrazolone derivative, is a tertiary amine and reacts rapidly with nitrogen oxides in the air to form NDMA. Another example is vasodilators derived from nitrate or nitrite esters. Because of their chemical properties, vasodilators, such as hydralazine and minoxidil, can behave as potential nitrosating agents and form certain types of nitrosamines depending on the chemical structures. Additionally, cimetidine, a histamine analogue and blocker of H_2 receptor, has been effectively used for the treatment of peptic ulcer, but this drug may be associated with nitrosation reactions (36). Fol-

lowing antihelmintic treatment with piperazine (1, 4-diazacyclohexane) in patients, piperazine-derived N-notrosamines were excreted in urine (37).

7) **Others:** In machine shops, sodium nitrite is used for rust inhibitor in synthetic cutting oils and contributes to the formation of DENA. Although DENA is not sufficiently volatile to cause inhalation exposure, it can affect dermal exposure to workers. Also, amine factories can be the source of NDMA exposure in the air. It is certain that a variety of nitrosamines can possibly be formed in many industries, given the use of amnies and their nitrosation.

2. Environmental Sources

In addition to industrial exposures, humans can be exposed to nitrosamines through environmental sources.

1) **Food**: Sodium nitrite has been used not only to prevent microorganisms in meats, but also to keep the reddish colour of meats under the fluorescent light. This preservative is also used for the preservation of many processed foods, such as ham, bacon, cheese, pickled fish and other smoked foods and cause the formation of nitrosamine. The amount of nitrosamine formed in cooked meats and processed foods is influenced by the method of cooking, cooking temperature and cooking time. The most common nitrosamine is NDMA in foods, but N-nitropyrrolidine (NPYR) is additionally found in fresh and cooked fish (38). In fried bacon, NDMA, NPYR and vegetables were occasionally contaminated with low levels of NDMA. Also, NDMA and DENA are detected in edible oils at concentrations as high as 23 and 28 ug/kg, respectively.

Considerable attention has been paid on the presence of nitrosamines in alcoholic beverages. Although rum, French apple brandies and cognac contain nitrosamines, beer and Scotch whiskey are the only significant sources of NDMA. In the process of beer production, the malt contains amine precursors including hordenine, gramine and methyltyramine is exposed to nitrogen oxides during the drying process and forms NDMA. However, the modification of drying process can reduce the levels of NDMA. Havery *et al.* (39) analysed 80 samples of imported beers and found NDMA ranged from 0.2 to 13 ug/liter, averaging 1ug/liter.

2) **Water:** Nitrosamines have been shown to be absent in drinking water in some states U.S., but NDMA has been observed in deionized water at concentrations less than 0.25 ug/kg (40). In industrial wastewater, volatile nitrosamines such as NDMA have been detected at concentrations ranging from 0.2 to 5 ug/liter. With high nitrate concentrations, well water also contains NDMA and DENA at levels lower than 0.01 ug/liter (41).

3) **Air:** NMOR and NDMA are found in the interior air of automobiles. The sources include rubberized mats, rubber grommets and rubber sealants from tire. However, airborne NDMA and other nitrosamines do not present a widespread air pollution problem, but they are localized because nitrosamines can be decomposed rapidily by light (23). In addition, nitrosamines in the indoor air are attributed largely to sidestream smoke from cigarette smoking.

4) **Cigarette Smoking**: Tobacco and tobacco smoke contain three types of nitrosamines; volatile nitrosamines, nitrosamines derived from residues of agricultural chemicals on tobacco and the tobacco specific nitrosamines (42-45). These compounds are formed during tobacco

processing and during smoking from the reactions between amines and nitrosating agents. Among the three types of nitrosamines, tobacco specific nitrosamines are of great interest because they are more potent in carcinogenesis than others and their levels are much higher (0.44-4.6 ug/cigarette). The tobacco specific nitrosamines include N'-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-butanone (NNK), N'-nitrosoanabasine (NAB), 4-(methylnitrosamino)-4-(3-pyridyl)-butanal (NNA) and N'-nitrosoanatabine (NAT) (46). NNN, NNK and NNA are formed from nicotine and NNN, NAB and NAT can be formed from nicotine, anabasine and anatabine respectively. It has been estimated that tobacco smoke contributes far more to human exposure to nitrosamine than any other source except occupational exposure. The levels of nitrosamines are generally greater in the sidestream smoke, which is generated from the butt end during a puff. Also, cigars can generate more nitrosamines than cigarettes. More importantly, the levels of tobacco specific nitrosamines are much greater than any other tumor-initiating agent in the particulate phase of tobacco smoke.

V. Mechanism of Nitrosamine carcinogenesis

1) Metabolic activation: Nitrosamines are indirect carcinogens and require metabolic activation to generate reactive, electrophilic species

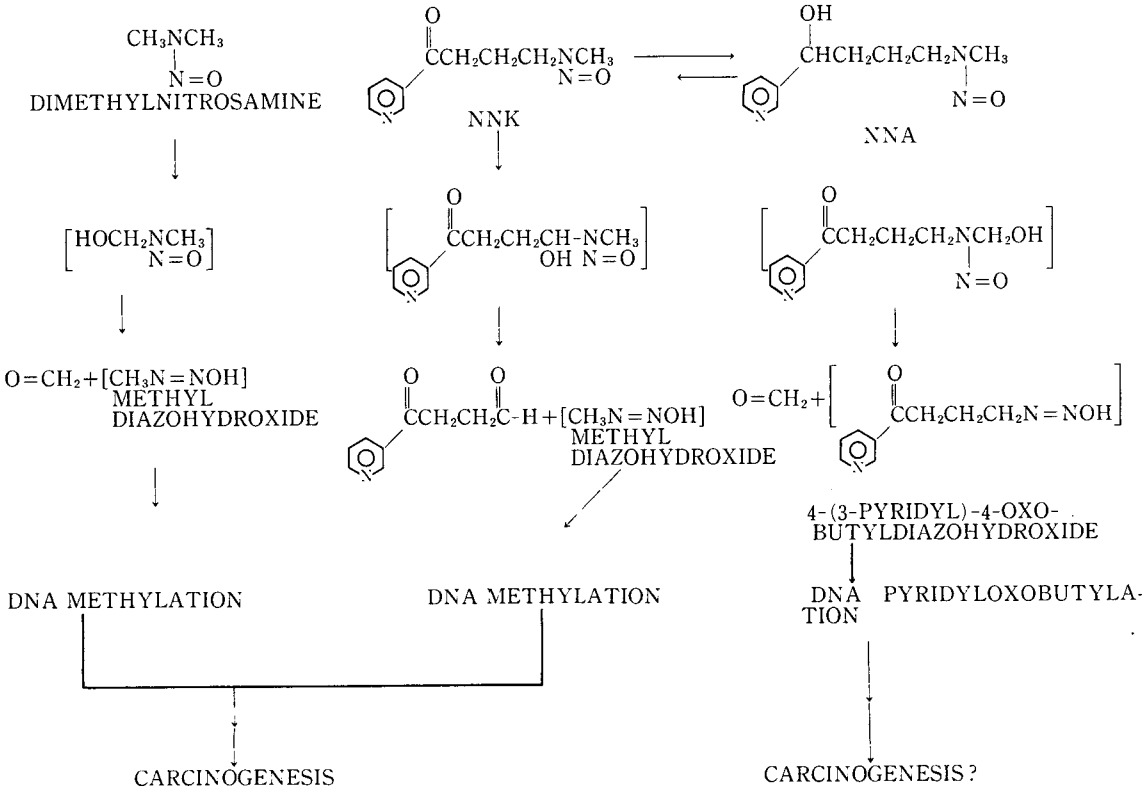


Fig. 1. Metabolic pathway of dimethylnitrosamine and NNK in chemical carcinogenesis (46).

that initiate a toxic response. Under the cyto chromal P-450 metabolizing system, nitrosamines undergo α-carbon hydroxylation and then chemically decompose to alkyldiazohydroxides. The alkyldiazohydroxides are the ultimate electrophilic species which are capable of covalently binding with celluar macromolecules including DNA, RNA and protein (46, 47). However, another metabolic pathways, possibly dependent on amine oxidase, may also be involved. Exceptionally, some tobacco specific nitrosamine, such as NNK can damage DNA either by methylation or by pyridyloxobutylation (Fig. 1.)

2) Macromolecular Interactions and Repair Mechanisms: Nitrosamines can alkylate any of N or O in all four bases of DNA, sugar residues and phosphates of the DNA backbone. The proportion of alkylation in vivo at each site is summarized in Table. 2 (48). As shown, N^7 position of guanine is the most susceptible site. However, alkylated DNA-derived guanine in the O^6 position is a likely cause of misparing and mutation since this position is involved in base pairing (49). O^6-methylguanine causes miscoding, leading to incorporation of thymidine during DNA replication, but it can be repaired by O^6-methylguanine-DNA methyltransferase (50).

Conditions that accelerate replication or delay repair could enhance miscoding, thereby increasing the number of mutation and enhancing the carcinogenic effects. On the other hand, conditions that delay replication or enhance repair efficiency would have the reverse effect. The repair mechanism is more efficient at the low dose exposure than at the high dose exposure (51). Furthermore, this repair mechanism is more efficient in humans than in other species.

There is also an evidence that K-ras gene was activated by GC to AT transition mechanism in codon 12 in 100% of NOMA- and NNK-induced lung tumor (52).

VI. Adverse Health Effects

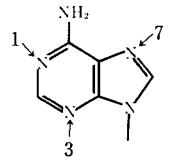
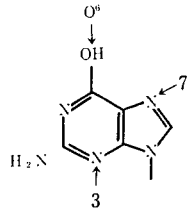
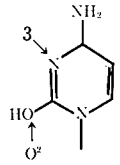
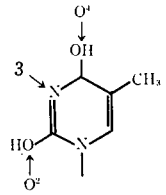
Exposure to nitrosamines has been implicated in the causation of variety of acute and chronic toxicities in animals and humans. The carcinogenicity, mutagenicity and teratogenicity have been demonstrated for nitrosamines and this has been also proven by bacterial tests. In human carcinogenicity, although the epidemiological studies suggested the strong relationship between nitrosamines and human cancers, sister-chromatid exchanges (SCE) in a S9-coupled human leukocyte culture system exhibited a dose-dependent twofold increase of SCE induction indicating good correlation between animal carcinogenicity of nitrosamines and SCE induction (53).

1) Animal Toxicity: It was first observed that NDMA strikingly induced acute hepatic toxicity in rodents (8). Generally, nitrosamines-induced acute toxicity in organs other than the liver is relatively uncommon and the hepatoxicity of nitrosamines follows a marked impairment of protein synthesis. However, renal toxicity is of particular interest since it appears to correlate with the late appearance of renal neoplasia in a manner somewhat similar to that in the liver.

The acute toxicity of nitrosoalkylamines decreases with increasing chain length, and at the LD_{50} level, DENA was a far more effective nasal toxin and carcinogen than was NDMA (54).

In chronic administration of nitrosation of nitrosamines, NDMA led to liver cancer with low levels, but shorter exposure (1 to 4 weeks) at

Table 2. The proportion of alkylation in vivo at each site of DNA base.

DNA base		position	% of total alkylation	
			methylation	ethylation
ADENINE:		N-1	0.8	0.1
		N-3	4	4
		N-7	1.5	0.6
GUANINE:		N-3	0.6	1.5
		O^6	3-6	8
		N-7	69	12
CYTOSINE:		O^2	0.1	2
		N-3	0.5	0.3
THYMINE:		O^2	0.1	7
		N-3	0.3	0.4
		O^4	0.1	2.5

* The arrows on DNA bases indicate modification sites (48).

higher dose induced kidney tumors (9). It is notable that 85% of the 209 nitrosamines tested have been shown to be carcinogenic in laboratory animals. Schmähl *et al.* (55) reported that DENA in 20 species of animals. There are species differences in relation to the tissue primarily affected. For example, DENA leads to liver cancer in rat or mice but lung cancer in hamster. In the comparative carcinogenic study of DENA and benzo (a) pyrene (B(a)P), the lung tumor incidence rate was much greater in the DENA (100%) than in the B (a) P (46%) (56). These tests have also revealed the importance of enhancers and inhibitors of carcinogenicity. For example, agents (iodide, thiocyanate) that promote cell proliferation in the liver enhance carcinogenicity, but inhibitors, such as ascorbic acid and vitamin E, inhibit carcinogenicity (57).

2) **Human Toxicities:** Many nitrosamines are clearly carcinogenic in every species of animals in which they have been tested and mutagenic in microbial and mammalian test system. Some

are teratogenic in hamster and rats. However, the value of these tests in the prediction of risk to humans is unknown. Evidence implicating nitrosamines in the development of human cancer is largely circumstantial. Nonetheless, epidemiological studies have suggested a possible association between exposure to high levels of nitrosamines and a high incidence of stomach cancer, esophageal, liver, nasopharyngeal and bladder cancer. It reported that Japan has the highest incidence of stomach cancer in the world. This high rate has been associated with the consumption of salted dry fish which may contain high levels of certain secondary amines and other precursors of N-nitroso compounds (15, 58). In addition to fish consumption, to drink well water, which contains high levels of nitrate, could affect the elevated nitrosamine exposure.

In England, Hill *et al.* (26) reported the correlated difference in stomach cancer mortality rates with the nitrate content of drinking water in two towns. In China, certain areas had a high incidence of esophageal cancer and this finding was associated with the consumption of nitrite-rich pickled vegetables (14, 59, 60). In salted vegetables, nitrosamines were detected and they were also associated with liver cancer (61). In contrast to geographical pattern of stomach or esophageal cancers, the liver mortality in China was also associated with high levels of nitrite and nitrate in the soil and nitrosamines were detected in salted vegetables, which were commonly eaten in areas with high rates of liver cancer. However, other important potential confounding factors, such as hepatitis B virus and aflatoxin, are suspected to be implicated as etiologic agents in this disease. Additionally, recent studies demonstrated that NNK and other tobacco specific nitrosamines could cause human cancer (46, 62-64). Presumably, this demonstration might be possible since tobacco specific nitrosamines were detected at greater levels in tobacco smoke than any other tumorigen and their carcinogenicity was more potential than other types of nitrosamines. Especially for cancers related to smoking, these specific nitrosamines should be considered as important chemicals for the cancer causation.

Ⅶ. Molecular Epidemiology of Nitrosamines

To evaluate the carcinogenecity of chemicals, we often rely on in vitro and in vivo tests.

Especially animal experiments can provide dose-response data and have been considered useful predictions for human responses, but it should be emphasized that regulatory agencies value epidemiological data as the most conclusive evidence for human carcinogenicity.

Cancer research in occupational or environmental setting has been greatly progressed by the introduction of molecular epidemiology which combines molecular toxicology and analytical epidemiology. The principle of molecular epidemiology is based on human monitoring of carcinogen exposure and investigation of preclinical responses in multi-stage of chemical carcinogenesis quantifying biomarkers such as specific carcinogen-DNA adduct, carcinogen-protein adduct, carcinogen metabolites and other genotoxical alterations in human tissues and body fluids.

A good example of molecular epidemiological study on N-nitrosamines was conducted in Lin-Xian Subjects, China (65). Lin-Xian was the highest esophageal cancer incidence area and the amounts of N-nitrosmino acids in urinary excretion were significantly higher in Lin-Xian

Subjects than those in Fan-Xian, where the incidence was relatively low.

In addition, the amount of O^6-methyl-2'-deoxyguanosine, DNA adduct presumably induced by nitrosamine exposure, were higher in DNA of esophageal or stomach mucosa of patients from Lin-Xian than that from Europe (Lyon and Essen).

These findings indicated that the elevated levels of N-nitrosamino acids in urine and O^6-methyl-2'-deoxyguanosine in esophageal DNA could be the result of a recent exposure to N-nitrosamines or reduced repair of DNA damage for nitrosamine carcinogenesis.

Ⅷ. Summary and Conclusion

Evidence of carcinogenicity provided by well-conducted experiments in animals and epidemiological studies indicate that nitrosamines are potentially carcinogenic in humans. Moreover, humans may be easily exposed to nitrosamines both in the industries and in the environment via inhalation, ingestion and dermal contact. The sources of nitrosamine exposure are associated with the manufacturing processes in the industries and with the contamination of nitrite or nitrate in the environment. Especially, the use of nitrites in the preservation of foods can increase the exposure of nitrosamines to humans. Consequently, the expected exposure levels of nitrosamines should be reduced to the safety for human health. The strategies for the reduction of nitrosamines may be recommended:

1. The manufacturing processes which are associated with the formation of nitrosamines should be modified either to reduce or to eliminate nitrosamines.
2. The use of nitrites as food preservatives should not be allowed legally after careful investigation of their risk assessment.
3. Any sources related to nitrosamine formation should be prevented: nitrogen fertilizer and structurally nitrosable chemicals.
4. The use of inhibitors such as vitamin C and others should be considered after further research on their preventive roles in nitrosamine formation.

The strategies mentioned above, however, should be regarded based upon human risk assessment. For the control of these compounds, legal standard limits should be made by law. Additionally, quitting smoking would decrease the exposure levels of potent tobacco specific nitrosamines and reduce the possible cancer incidence in both active smokers and passive smokers.

Based on animal experiment and limited epidemiological study results, we postulate that nitrosamines might be one of major contributing carcinogens for the high incidence of stomach and liver cancer in Korea. To prove the postultion molecular epidemiological study of nitrosamine should be carried out in the future.

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