

# **N-OXIDES AND NITROSAMINES: THEIR ENVIRONMENTAL IMPLICATIONS**

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The concentration of  $N_2O$  has risen steadily. There are many sources for its formation. It gives rise to  $NO$ ,  $NO_2$  and eventually to  $HNO_3$ .  $NO_2$  along with  $NO$  is a common pollutant. The two together can give dinitrogen trioxide ( $N_2O_3$ ) and  $NO_2$  on dimerisation gives dinitrogen tetraoxide ( $N_2O_4$ ). Pathways for the nitrosation of primary, secondary and tertiary amines are fairly well understood. Kinetics of nitrosation of many secondary amines have been worked out.

Nitrosation of food constituents has attracted considerable attention. This has led to changes in curing of bacon and modifying processing of barley malt for beer production, resulting in reduction in N-nitrosodimethylamine (NDMA) levels. Many nitrosamines have been identified from tobacco. Efforts have been made in recent years to correlate risk to human cancer from exposure to N-nitroso compounds. Also, some understanding of metabolism and mode of action of N-nitroso compounds has been obtained.

Though sensitive and reliable methods of analysis for nonvolatile nitrosamines have been developed, yet the situation regarding the analysis of volatile nitroso compounds is far from satisfactory.

## **INTRODUCTION**

In addition to the possible long term and secondary effects of the oxides of nitrogen on the ecosystem, compounds like nitrates, nitrites, amines and nitrosamines have profound effects on the living systems.

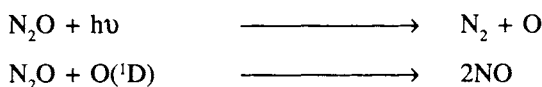
Nitrosamines arise generally from the interaction of nitrous acid with secondary or tertiary amines. Both amines and nitrosating agents are widely distributed in the environment. Many bacteria are capable of reducing nitrate to nitrite. Many types of nitrosatable amines including amino acids are present in the environment. Nitrosation can take place in the human stomach and compounds like thiocyanate can enhance the nitrosation reaction by a few orders of magnitude.

Nitrosamines have been known since nineteenth century but have come into sharp focus ever since Magee and Barnes (1956) showed that nitrosodimethylamine

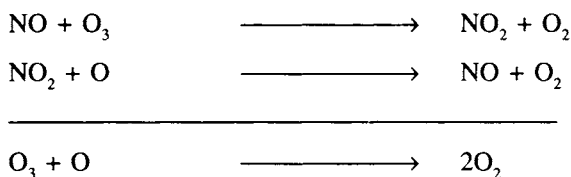
is a potent carcinogen. Besides the occurrence of nitrosatable amines in some food items, tobacco has been found to be a serious source of nitrosamines. Today there is vigorous effort related to the occurrence, formation, analysis, chemistry, metabolism, mode of action and biological activity of N-nitroso compounds. A resume of these efforts is presented.

## N-OXIDES

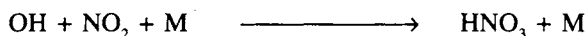
The concentration of  $N_2O$  has been rising steadily and was estimated at 303 ppb in 1984 providing with an atmospheric burden of  $1500 \times 10^{12}gN$ . The sources include oceans, combustion of fossil fuels, fertilized agricultural fields, tropical and subtropical forests and woodlands. The principle sinks are photolysis and reaction with  $O(^1D)$  as follows:



$N_2O$  diffuses upwards from troposphere to stratosphere to give NO as shown above. NO so formed reacts with ozone as below :



The reaction of  $NO_2$  with OH is as follows :



$HNO_3$  is washed out of the troposphere. A major portion of  $O_3$  destruction takes place through this system. Oxides of nitrogen are also involved in the following reactions:



Nitrogen dioxide ( $NO_2$ ) is a common pollutant along with nitric oxide (NO) by combination process. The two together on combination can give dinitrogen trioxide ( $N_2O_3$ ).

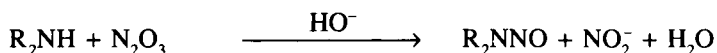


Nitrogen dioxide can also undergo dimerisation to dinitrogen tetroxide:

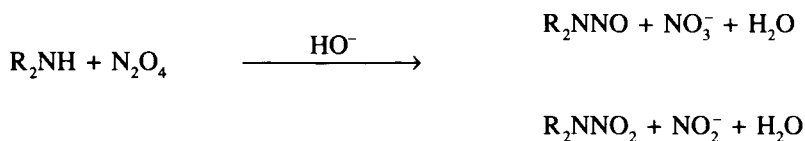


These reactions are more favoured in solution than in the gaseous phase. Thus  $\text{N}_2\text{O}_3$  and  $\text{N}_2\text{O}_4$  could accumulate on particulate matter and in polluted atmosphere.

At high concentrations  $\text{N}_2\text{O}_3$  ( $\rightleftharpoons \text{NO} + \text{NO}_2$ ) react with amino compounds in solution to produce N-nitrosamines (Challis and Kyrtopoulos, 1978, 1979) :



where  $\text{N}_2\text{O}_4$  ( $\rightleftharpoons 2 \text{NO}_2$ ) gives a mixture of N-nitrosamines and N-nitramines.



It has been shown that N-nitrosamine formation from nitrogen oxides has considerable free radical character. Challis *et al.* (1982) reacted N-methylalanine with gaseous  $\text{NO}_2$  and observed the formation of a number of products resulting from nitrosation and nitration as given in Fig. 1.

Significant amounts of N-nitrosamines are obtained even when dilute gaseous  $\text{NO}_2$  (5 ppm) reacts with heterocyclic amines (such as morpholine, pyrrolidine, etc.). Since the dimerization of  $\text{NO}_2$  to  $\text{ON-ONO}_2$  in aqueous solution is a facile reaction even at low concentration of  $\text{NO}_2$ , the existence of lower limit below which N-nitrosamine formation can be ignored therefore seems unlikely according to Challis *et al.* (1982).

## N-NITROSATION

N-Nitroso compounds are produced by the acid-catalysed reaction of nitrite with certain nitrogen compounds. N-Nitroso compounds are divided into N-nitrosamines derived from dialkyl, alkylaryl, diaryl or cyclic secondary amines and the nitrosamides derived from N-alkylureas, N-alkylcarbamates and simple N-alkylamides. N-Nitroso derivatives can also be made from cyanamides, guanidines, amidines, hydroxylamines, hydrazines and hydrazides.

MECHANISM AS PROPOSED BY CHALLIS et al ,1982

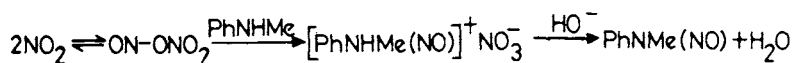
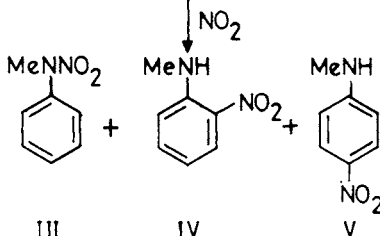
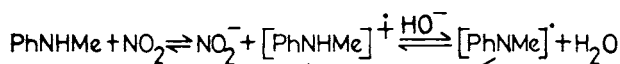
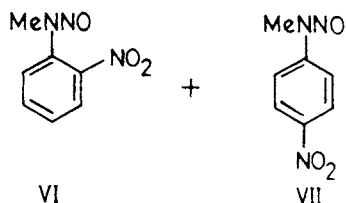
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Fig. 1. Two different reagents responsible for nitrosation and nitration.

Possible pathways for the formation of nitrosamines from secondary, tertiary and primary amines are given in Fig. 2. Nitrosation of the secondary amines is of importance due to their occurrence in food, especially after fermentation and cooking. Fish contains relatively large amounts of dimethylamine. Some secondary amines are also used as drugs and pesticides.

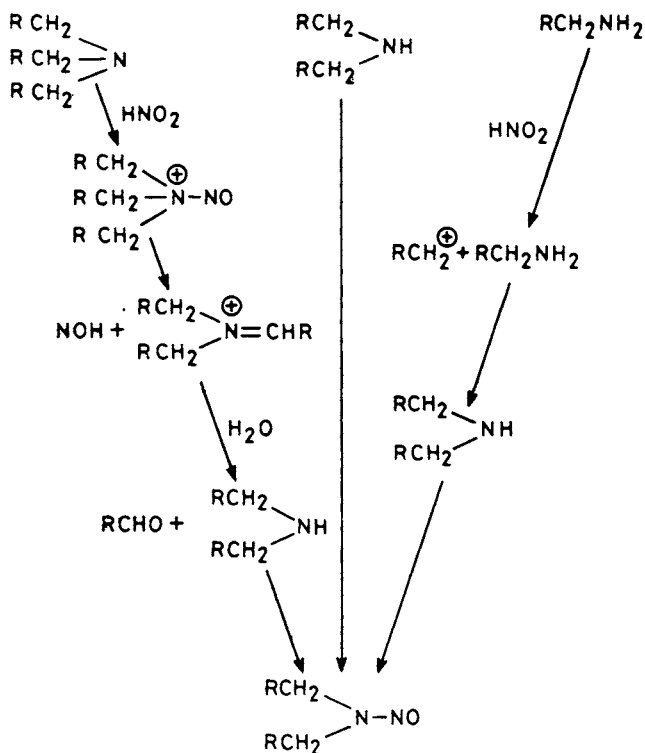
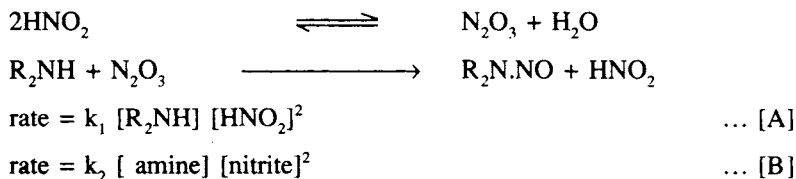


Fig. 2. Proposed pathways for the formation of a nitrosamine from a secondary, tertiary and primary amine (Scanlan, 1975).

Most secondary amines are nitrosated according to the following equations:



Nitrosating agent  $\text{N}_2\text{O}_3$  is produced from two molecules of  $\text{HNO}_2$ , the rate of nitrosation is proportional to  $[\text{amine}]$  and  $[\text{N}_2\text{O}_3]$  i.e.  $[\text{HNO}_2]^2$

In [A] [nonionized  $\text{R}_2\text{NH}$ ] and free  $[\text{HNO}_2]$  are used and  $k_1$  should be independent of pH, but  $[\text{R}_2\text{NH}]$  and  $[\text{HNO}_2]$  have to be calculated for each pH. The equation [B] is more appropriate where rate constant  $k_2$  varies with pH although the total concentration of amine and nitrite are used irrespective of species involved. The reaction rate  $k_1$  and  $k_2$  show maximum value at pH 3.4 (pka of  $\text{HNO}_2$ ).

Mirvish (1975) has given the rate constants  $k_1$  and  $k_2$  of a large number of secondary amines. As  $k_1$  values varied only times 34, showing that the nonionized species of the amines had somewhat similar reactivities towards  $N_2O_3$ . Therefore if  $k_1$  were constant,  $k_2$  would be a function of  $pK_a$  of the amine and from [A] and [B] above the following expression would emerge:

$$k_2 = \frac{k_1 [R_2NH] [HNO_2]^2}{[\text{amine}] [\text{nitrite}]^2}$$

Thiocyanate, bromide and chloride increase the rate of nitrosation. The order of activity is  $NCS^- \gg Br^- > Cl^-$ . In presence of thiocyanate, the rate of nitrosation of morpholine, N-methylamine, aminopyrine and sarcosine were found to increase.

Tertiary amines may be nitrosated under mild acidic conditions in low yields and also give corresponding aldehydes and ketones. Singer (1980) used tri-n-butylamine (TnBA) for his studies and determined the products using GC-MS as given in Fig. 3.

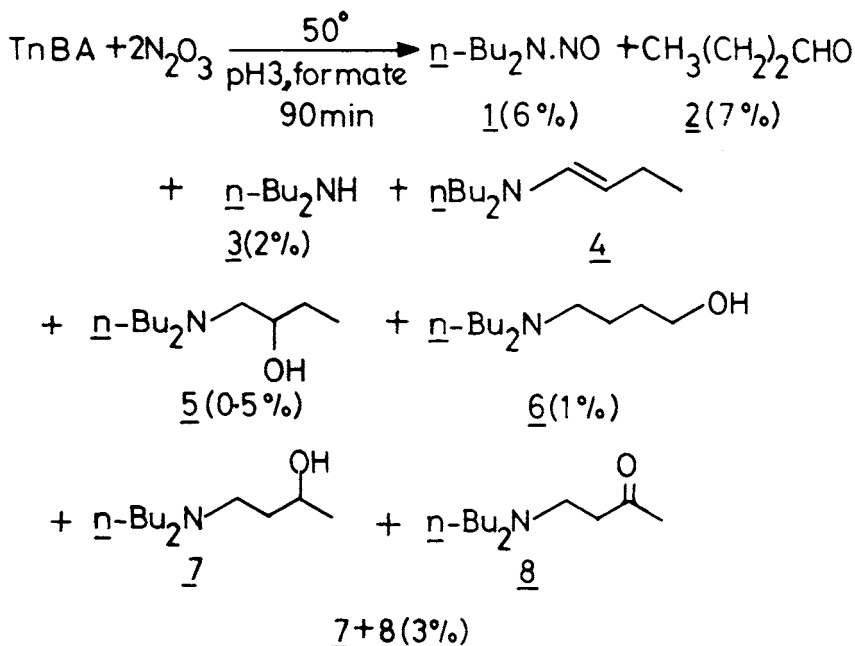
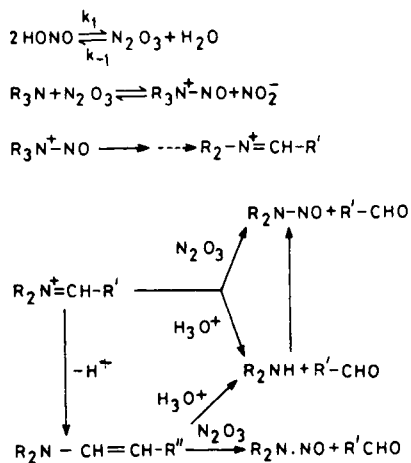


Fig. 3. Products of nitrosation of tri-n-butylamine (Singer, 1980).

A summary of the possible reaction in which N-nitroso ammonium ion is the likely intermediate is given below :



The occurrence and distribution of alcohols and the ketone (5-8) (Fig. 3) have been rationalised by "ω-1" radical cation mechanism by Singer (1980) as shown in Fig. 4.

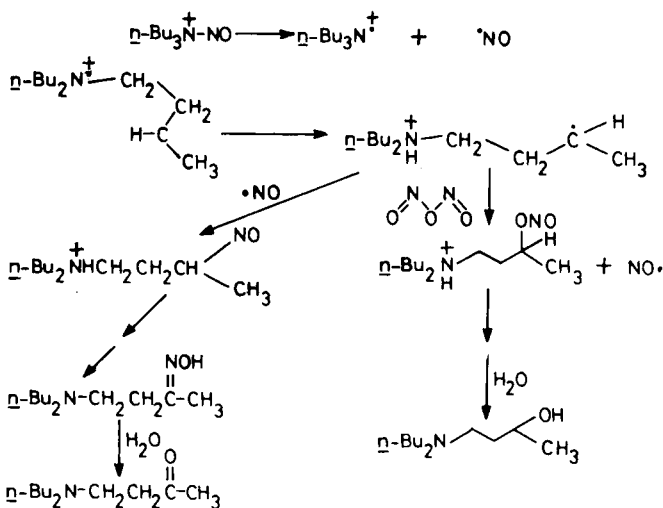


Fig. 4. Possible mechanism of formation of oxygenated products via "ω-1" radical-cation pathway.

## FORMATION OF NITROSAMINES IN ORGANIC SOLVENTS

The formation of nitrosamines in aqueous solution has been well established. Iitsuka *et al.* (1985) have studied the formation of nitrosamines in organic solvents as organic solvents are frequently used in food processing, cosmetics and drugs. The organic solvents used by them were chloroform, benzene, ethyl acetate, n-hexane, acetonitrile, acetone and ethyl alcohol.

Their results indicated that the level of nitrous acid in aqueous systems containing organic solvents was higher than that in the control at pH 2-5. Acceleration of the formation of the nitrosamines in the aqueous system containing organic solvents may be due to the increase in available nitrous acid level in the pH range. It was concluded that most organic solvents suppress ionization of nitrous acid and accelerate the nitrosation of secondary amines. The secondary amines also dissolved readily in organic solvents as the unprotonated forms which are the reactive forms for nitrosation. Suppression of protonation of the amines in non-aqueous solvent systems or aqueous systems containing organic solvents may also have contributed to the accelerated formation of the nitrosamines.

## FORMATION AND OCCURRENCE OF NITROSAMINES IN FOOD

In foods, the nitrosation of food constituents usually takes place through nitrous anhydride, which is formed from nitrite in acidic, aqueous solution. In some cases nitrosodimethylamine formation takes place as a result of direct fire drying process. In this case, oxides of nitrogen in the drying air nitrosates amines in the food being dried.

Nitrosodimethylamine (NDMA) is the most commonly occurring volatile nitrosamine while nitrosopyrrolidine (NPY) occurs to lesser extent. Cured meats, cooked bacon, beer, some cheeses, nonfat dry milk and fish have been shown to contain nitrosamines. The volatile nitrosamines generally occur in the  $\mu\text{g}/\text{kg}$  range. There are possibly several hitherto undetected volatile nitrosamines present in food materials and large amounts of nonvolatile nitrosoamines.

Of the various N-nitroso compounds, N-nitrosamines have been studied most extensively in foods and reviewed by Scanlan (1983). In 1956, Magee and Barnes described the induction of liver tumor in rats by feeding NDMA. During the last 33 years a large number of nitrosamines have been tested for their biological effects and found to be carcinogens. This has been a cause for continuous concern.

As has been discussed before the rate of nitrosation is pH dependent and is dependent on the total amounts of nitrite and amine as shown below:

$$\text{Rate} = k_1 [\text{amine}] [\text{nitrite}]^2$$

Since the unprotonated amine reacts with the nitrosating agent the rate of nitrosation for a secondary amine is inversely proportional to amine basicity. Morpholine ( $\text{p}K_a$  8.7) forms nitrosamine considerably faster than does diethylamine



( $pK_a$  10.7) at the same pH. The pH optimum for most secondary amines for nitrosation is between 2.5-3.5. The range is sufficiently close to the acidity of the human stomach to allow nitrosation to proceed there.

The chemistry of food is complex as the composition of food constituents is very complicated. Nitrosation of food constituents is affected by the concentration and nature of nitrosatable substances, the concentration and type of accelerators and inhibitors and factors such as time, temperature, pH, etc.

The picture is further obscured as adequate analytical methodology exists only for volatile nitrosamines but not for nonvolatile ones. Although methods are available for the determination of a few nonvolatile nitrosamines, but generally analytical methodology for nonvolatile nitrosamines and other N-nitroso compounds is still in early stage of development.

Some data as compiled by Scanlan (1983) on some food materials will be summarized in the following:

### **Cured meats**

Since sodium nitrite is used for curing meats and sometimes sodium nitrate is also added, this category of food has received much attention and also cooked bacon has been subject of much investigation. In cooked bacon NPYR, and usually NDMA and N-nitrosohydroxypyrrolidine (NHPYR) are the most consistently occurring nitrosamines. Some of these levels have been compiled by Gray (1981). Much of the volatile nitrosamines (60-95% of total NPYR and 95-100% of NDMA) are lost in the fumes when bacon is fried (Gough *et al.*, 1976). With restriction on the levels of nitrite and increased amounts of ascorbate in bacon curing in the United States, the NPYR levels have decreased from 1971 onwards. Cured meats other than bacon contain NPYR, NDMA, N-nitrosodiethylamine (NDEA), N-nitrosopiperidine (NPIP), N-nitrosomorpholine (NMOR) and N-nitrosoproline (NPRO). Though the levels of these nitrosamines are less than 1  $\mu\text{g}/\text{kg}$  when present.

### **Dairy products**

A number of cheeses and dried dairy products have been shown to contain low levels of NDMA. It is, however, intriguing why some cheese samples contain NDMA and others not.

### **Fish**

As many species of salt fish contain considerable amounts of dimethyl and trimethylamines, fish has been a subject of much examination for volatile nitrosamines. Gough *et al.* (1978) reported NDMA levels of 1-10  $\mu\text{g}/\text{kg}$  in over 1/3rd of nearly 60 samples of uncooked fish examined by them. Some of the samples of cooked fish did not contain NDMA perhaps due to the volatility of the compounds. Nevertheless, the mode of cooking can influence the NDMA content (Matsui *et al.*

1980). In their studies Matsui *et al.* showed that NDMA content in fact increased during broiling by a factor of 2-3, possibly due to the interaction with oxides of nitrogen produced from the gas range.

## Beer and Malt

The presence of NDMA in beer has been reported by various workers. Mangino *et al.* (1981) have summarized NDMA levels found by workers in different parts of the world. The source of NDMA in beer is barley malt, wherein it is produced from reaction of oxides of nitrogen and barley malt alkaloids during the direct- or fire-drying of malt. By modifying the firing kilns to indirect ones and by the use of sulphur dioxide during drying, considerable reduction in the NDMA levels has been achieved. The nitrosatable amines which occur in barley malt have been studied by Mangino *et al.* (1981) and Mangino and Scanlan (1982). Both hordenine and gramine are biosynthesised in green malt during fermentation. Possible mechanism for the formation of NDMA from these two alkaloids during direct firing process are given in Fig. 5 (Scanlan, 1983).

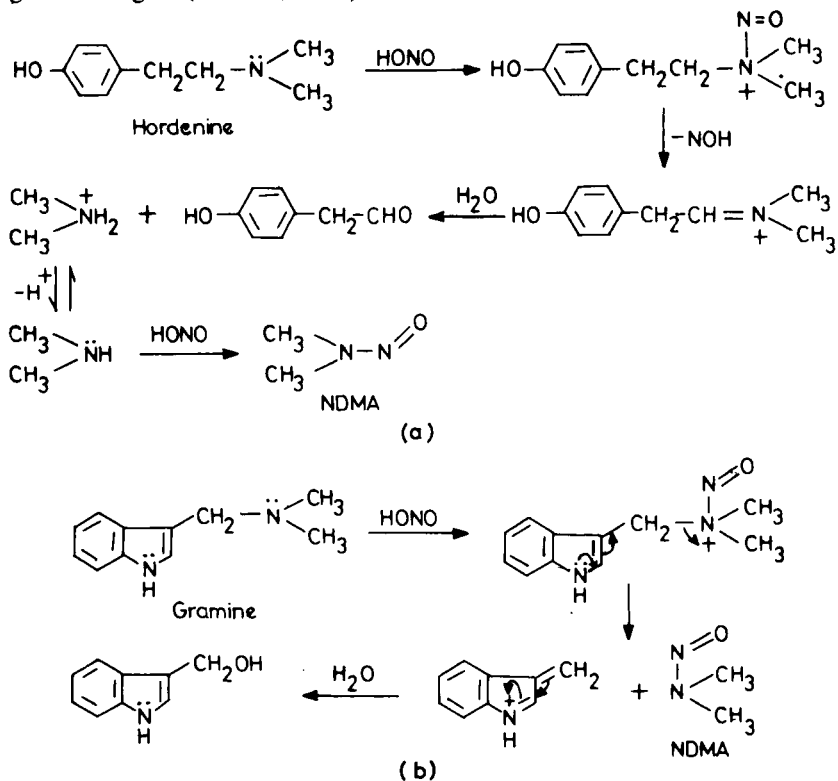


Fig. 5. (a) NDMA Formation from hordenine via nitrosative dealkylation and (b) Possible mechanism for NDMA formation from gramine (Scanlan, 1983).

### Baby bottle rubber nipples

In the recent examinations of baby bottle rubber nipples and pacifiers (Preussmann *et al.*, 1981), the presence of several nitrosamines like NDMA, NDEA, N-nitrosodibutylamine (NDBA), NPIP and 1-nitrosomethyl phenylamine (NMPHA) was observed. The concentrations ranged from 1-230  $\mu\text{g}/\text{kg}$ . The source could be vulcanizing retardants and nitrosamines in contaminated air.

From a recent compilation of estimated exposures of humans to nitrosamines in the United States by the National Academy of Sciences in 1981, Scanlan (1983) has adapted the data (Table 1). From Table 1 it is clear that besides food there is a large exposure to a variety of nitrosamines from cigarette smoking.

**TABLE 1: Estimated Relative Exposure of U.S. Residents to Volatile N-Nitroso Compounds (Scanlan, 1983)**

Nitrosamine*	Source	Route	Daily exposure ( $\mu\text{g}/\text{person}/\text{day}$ )
SEVERAL	Cigarette smoking	Inhalation	17
NDMA	Beer	Ingestion	0.3 - 0.97
NDMA, NMOR, NDEA	Automobile interiors	Inhalation	0.2 - 0.5
NDELA	Cosmetics	Dermal	0.41
NPYR	Cooked bacon	Ingestion	0.17
NDMA	Scotch whiskey	Ingestion	0.03

\* NDMA = N-nitrosodimethylamine;  
 NMOR = N-nitrosomorpholine;  
 NDEA = N-nitrosodiethylamine;  
 NDELA = N-nitrosodiethanolamine;  
 NPYR = N-nitrosopyrrolidine.

### Tobacco specific nitrosamines

In recent years there has been much activity in identifying N-nitrosamines from tobacco. Nicotine and minor nicotine alkaloids are considered to be the major habituating agents in tobacco products. They also give rise to carcinogenic, tobacco specific N-nitrosamines (TSNA). Some of these nitrosamines have been characterised in tobacco and/or tobacco smoke (Fig. 6). The most potent carcinogens amongst these are N-nitrosornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)butanone (NNK). N-Nitrosamines were detected, characterised and quantified in the commonly used chewing tobacco in India (Bhide *et al.*, 1985) and in the saliva of the users (Sipahimalani *et al.*, 1984). According to Hoffmann *et al.* (1989) the levels of NNN and NNK in chewing tobacco amount to 0.67-8.2 ppm and 0.1-0.4 ppm; those in Snuff range between 0.01-135 ppm and 0.1-13.6 ppm respectively.

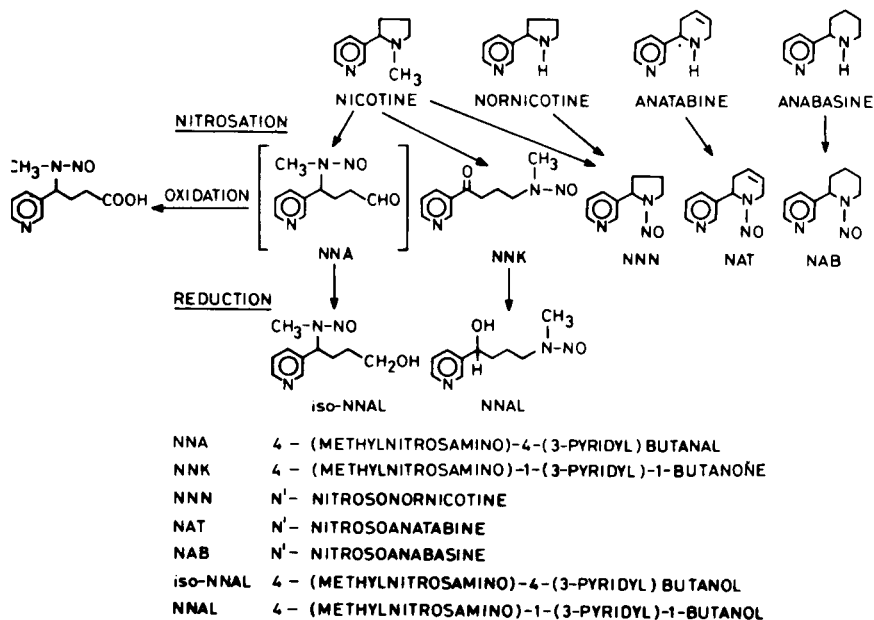


Fig. 6. Nitrosation of nicotine.

These amounts exceed by at least 2 orders of magnitude the levels of carcinogenic N-nitrosamines in other consumer products.

In beetle quid with tobacco, both TSNA and Areca-derived nitrosamines are formed. The chemical nature of nitrosamines from arecoline are given in Fig. 7. These are nitrosoguvacoline (NG) N-nitrosoguvacine (NGC), 3-(methylnitrosamine) propionaldehyde (MNPA) and the powerful carcinogen 3-(methylnitrosamine) propionitrile (MNPN). Their concentration in saliva of beetle quid chewers is reported to contain 1.2-38 ppb (NNN), 1.0-2.3 ppb (NNK), 2.2-350 ppb (NG), 0.0-30.4 ppb (NGC) and 0.5-11.4 ppb MNPN (Hoffmann *et al.*, 1989).

In studies carried out on Indian tobacco (Tricker *et al.*, 1989) for N-nitroso compounds, a number of volatile nitrosamines viz NDMA, NMEA, NDEA, NDPA, NDBA, NPIP, NPYR, NMOR, nonvolatile nitrosamines and tobacco-specific nitrosamines viz. NAB, NAT, NNN, NNK and iso-NNAL have been determined in a variety of tobacco products including chewing tobacco, *zarda*, *kiwam*, *masheri*, *bidi*, chutta and pipe tobacco. The estimated consumption figures on daily usage basis of some tobacco products are given in Table 2.

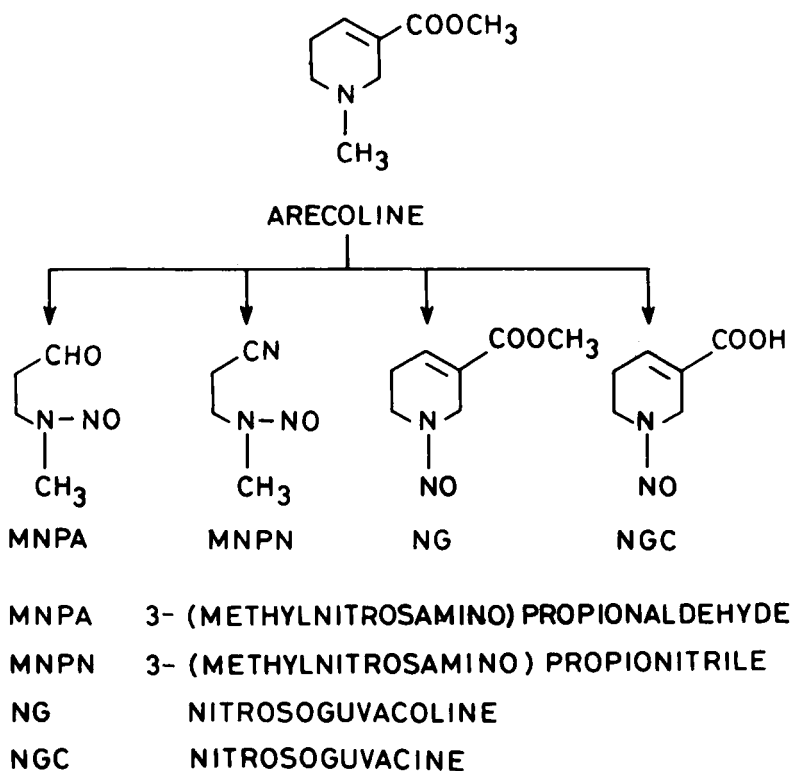


Fig. 7. Areca derived nitrosamines.

**TABLE 2: N-nitroso Compounds in Some Indian Tobacco Products**  
(Tricker *et al.*, 1989)

Tobacco type	Average usage (g/day)	Mean daily exposure (µg/day) Total NA	NNN & NNK
<i>Zarda</i>	10	405.3	174.5
<i>Kiwam</i>	3	36.0	14.6
<i>Masheri</i>	2	15.0	3.9

NA = Nitrosamines;  
 NNN = Nitrosonornicotine;  
 NNK = 4-(methylnitrosamino)-1-(3-pyridyl) butanone.

## RISK OF HUMAN CANCER

Surveys have been carried out in some countries to estimate the average per capita exposure to N-nitroso compounds (Forman, 1987). In Table 3 estimates from a few countries are given. The present intake is likely to be sufficiently lower as many changes in manufacture of bacon and beer have taken place lately. Nevertheless, it is now apparent that many food items contain unidentified N-nitroso compounds. This is due to the fact that improved methods are now being developed to determine

**TABLE 3: Estimate of Daily Intake ( $\mu\text{g}$ ) of Volatile N-nitrosamines in Different Countries (Forman, 1987)**

Country	Estimate	Major sources	Reference
UK	0.53*	Cured meats (81%)	Gough <i>et al.</i> (1978)
Japan	2.26	Dried fish (72%)	Maki <i>et al.</i> (1980)
Germany (FRG)	1.23	Beer (57%) Cured meats (17%)	Spiegelhalter <i>et al.</i> (1980)
Holland	1.0	Beer (90%)	Stephany and Schuller (1980)

\* Beer not included.

the total concentration of nitroso compounds in foods, including both volatile and nonvolatile compounds. Some of the hitherto unidentified compounds are likely to be nonvolatile nitrosamides which are often extremely unstable and likely to be broken down during cooking. Until constituent compounds can be isolated, chemically characterised, and biologically tested it will not be known whether or not these are carcinogenic. However, some correlations have been made from the available evidence. Association between consumption of beer and oesophageal, colorectal and renal cancers has been established. Human epidemiological studies have concentrated largely on the role of N-nitroso compounds in relation to oesophageal and gastric cancers. There is some evidence for an increased risk of stomach cancer associated with consumption of nitrite cured meat. There is also some evidence for the risk factor when salt pickled vegetables and salted fish are consumed by Chinese and Japanese populations. Specific mutagenic N-nitroso compounds, especially non-volatile nitrosoamides have been isolated from items like pickled Chinese cabbage.

The risk from endogenous formation of N-nitroso compounds can be of equal importance to that from the exogenous N-nitroso compounds. The two main precursors, nitrosating agents and nitrosatable amino substrates are both likely to be found in abundance in the diet. Nitrite occurs in certain cereal products, root vegetables, non-volatile beverages and as a curing agent in meats and some cheeses. Nitrite occurs principally in vegetables like lettuce, beetroot and spinach, in contaminated drinking water and from its use as curing agent. Nitrate acts as a nitrosating agent after its reduction to nitrite by bacterial microorganism found normally in the saliva and

in ingested gastric juice. It is estimated that nearly 5 percent of ingested nitrate is converted into nitrite in this way. In many countries, during past few decades the nitrate concentration of certain vegetable crops as well as nitrite levels of drinking water supplies have been on the increase. This has resulted due to the extensive use of inorganic fertilizers from which the nitrate can be leached and run off into surface and ground water and also absorbed by crops.

As is known, there is a wide variety of food items containing amino compounds and of these secondary amines and alkyl amides are the most likely substrates for nitrosation. Many types of fish are rich in these substances as also are pork-based products, coffee, tea, beer, wine, evaporated milk and certain spices. There is, however, no *a priori* reason to expect that simple cause and effect relationship between the presence of a precursor and induction of cancer exists. It is known for instance that diets with a high level of nitrate like vegetables may also contain protective and anticarcinogenic agents. Vitamin C can act as a protective agent as it acts as a competitive substrate for nitrite. Other inhibiting agents can be tocopherol, indoles, tannins and other polyphenolic compounds. Shephard *et al.* (1987) have reviewed the assessment of the risk of formation of carcinogenic N-nitroso compounds from dietary precursors in the stomach and have concluded that ureas and aromatic amines present potentially important risk factors in gastric cancer. The risk contribution of amines (including the proteins), guanidines, primary amino acids is uncertain. The risk factor due to alkylamines (primary and secondary), secondary amino acids is most probably negligible. They have recommended that for future investigation, the sources and levels of arylamines and ureas in the diet should be studied comprehensively and the carcinogenic potencies of key nitrosated products should be determined more precisely. Shephard *et al.*, (1987) have given estimates of relative risk posed by various nitrosatable precursors as shown in Fig. 8.

## METABOLISM AND MECHANISM OF ACTION OF N-NITROSO COMPOUNDS

It is well established that N-nitroso compounds are not active as such, but require *in vivo* activation. The formation of an alkylating electrophile to interact with nucleophiles in DNA (and other biopolymers) to form stable adducts such as alkylated purines and pyrimidine bases appears to be a requirement. The alkylation of genetic material is probably the initiating event in carcinogenesis. The important role of  $\alpha$ -hydroxy-N-nitrosoamines as proximate carcinogens (Fig. 9) has been confirmed by chemical and biological investigations.

A summary of present knowledge of human exposure to environmental N-nitroso compounds has been attempted by Preussmann (1984), and is given in Fig. 10. Although a qualitative picture does emerge, yet quantitation of endogenous exposure for risk estimation has not been possible so far. However, some recent developments may serve as a breakthrough in this area.

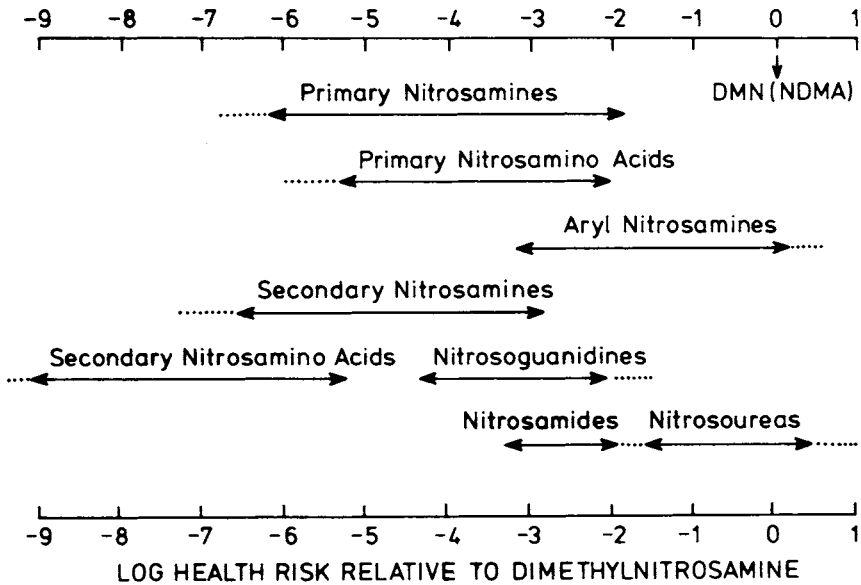


Fig. 8. Relative risk posed by dietary intake of nitrosatable precursors as compared to risk from dietary intake of dimethylnitrosamine (→Shows high nitrite concentration; ←shows low nitrite concentration; and . . . shows low pH) (Shephard *et al.*, 1987).

## ANALYTICAL PROCEDURES

Sen and Kubuacki (1987) have recently reviewed the methods that are available for the determination of total N-nitroso compounds, N-nitrosamides, N-nitrosamino acids and a variety of other nonvolatile N-nitroso compounds in foods and beverages. They have divided the methodologies into two broad categories: (a) methods for volatile N-nitrosoamines (VNA) and (b) methods for nonvolatile N-nitroso compounds (NVNC). Though the methodologies for VNAs have been well developed, but the situation with respect to NVNCs is not very satisfactory.

Hotchkiss (1981) has reviewed the analytical status with respect to the volatile N-nitrosamines. The VNAs can be extracted or distilled from the sample and after



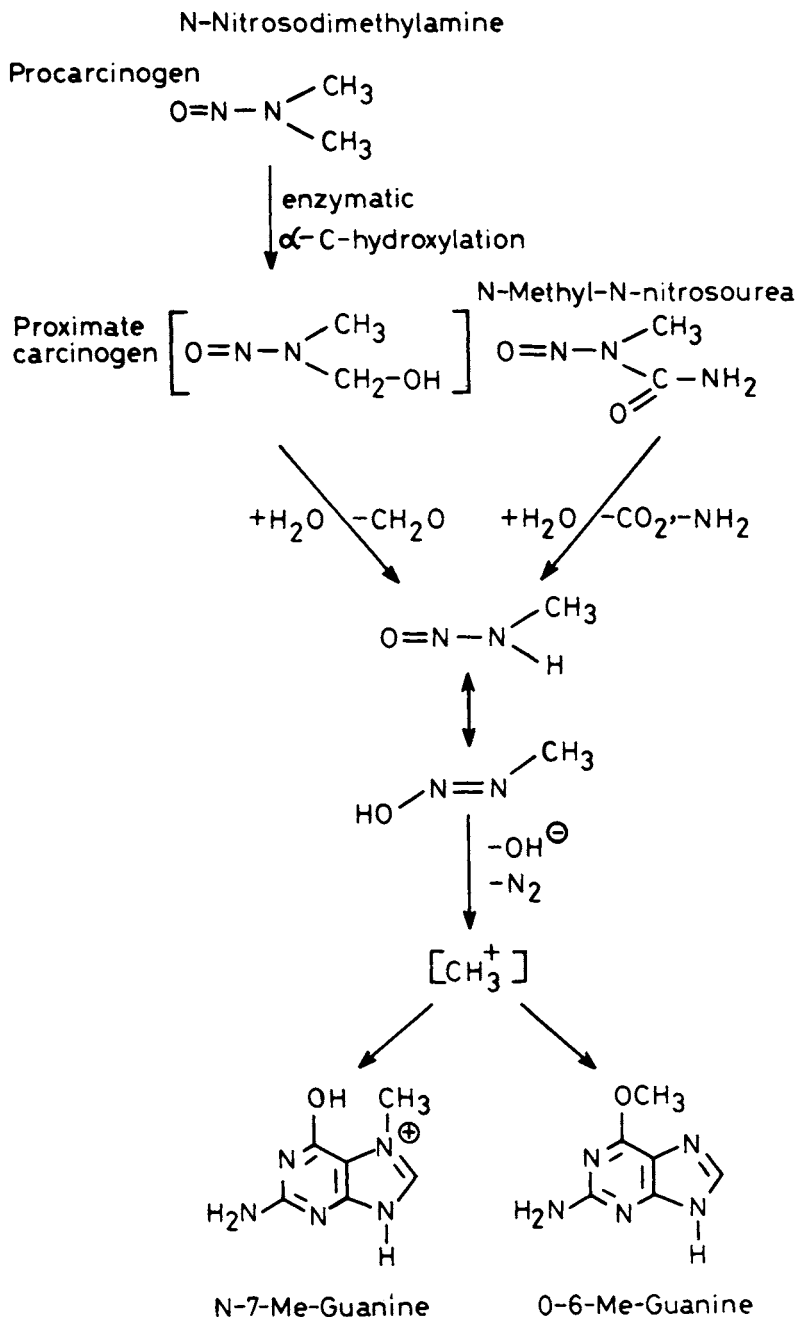


Fig. 9. Metabolism of N-nitroso compounds.

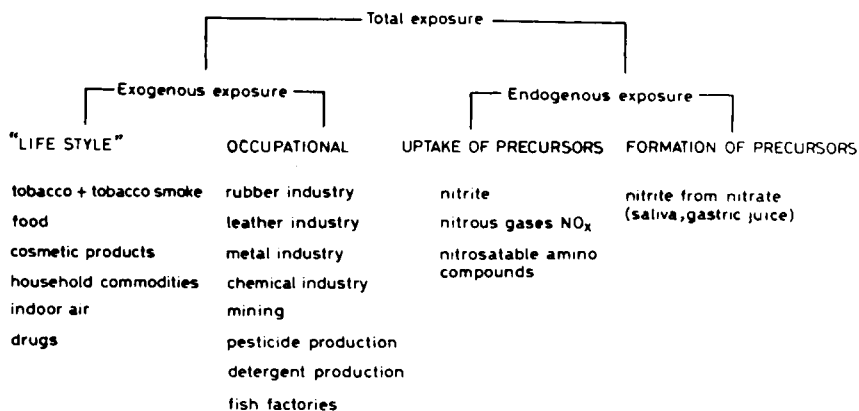


Fig. 10. Environmental N-nitroso compounds and human exposure.

suitable cleanup and concentration can be submitted to separation techniques. Chromatography has been used for quantitative and qualitative estimation and identification on VNAs. Use of TLC has been made for separation and densitometry of the fluorescent derivatives of VNAs in cooked bacon described for quantitative estimation. Gas chromatography is the method of choice for the separation of volatile NAs. In addition to packed columns, open tubular columns have been used most often in conjunction with mass spectrometry. The use of fluorescent derivatives of the amine produced by nitroso group cleavage has been made to detect and quantitate NAs in foods. Derivatisation has also been used to yield nonvolatile NAs amenable to gas chromatography.

The flame ionisation detector has found limited use in the determination of NAs in food because of lack of specificity. Alkali flame ionisation detector (AFID) or Coulson electrolytic conductivity detector (CECD) have also been used as a selective detector for volatile NAs in foods. NAs themselves are not amenable to electron capture detection but can be derivatised to electron-capturing species. Commercially available thermal energy analyser (TEA) is highly selective for N-nitroso compounds. TEA can be used as a detector for both gas chromatography (GC-TEA) or liquid chromatography (HPLC-TEA).

For TEA, N-nitroso compounds after exiting from chromatographic column, enter a flash heater and a catalytic pyrolysis chamber where the relatively weak N-N bond is ruptured. The nitric oxide produced is swept through a cold trap ( $-150^{\circ}\text{C}$ ) where organic compounds are frozen out. The emerging nitric oxide is made to react with  $\text{O}_3$  in a small chamber to produce an energy emission in the near infrared. This emission is detected by a photomultiplier tube, amplified and recorded. The response is proportional to N-nitroso group concentration in the original sample. The

GC-TEA system has been compared with GC-CECD, TLC and GC-MS for determination of volatile NAs in fishmeal and fried bacon by Fine *et al.* (1976). GC-TEA was found to be at least 50 times more sensitive and warranted less cleanup of the sample while GC-TEA give excellent analytical data, yet mass spectrometry (MS) has been considered the most reliable procedure for confirming the presence and identity of NAs. The maximum sensitivities reported for MS confirmation of NAs in food range over 4 orders of magnitudes.

The analytical methodologies for VNA have been well developed as discussed above but the situation with respect to methodologies on NVNCs is not quite as satisfactory.

In principle any nonvolatile compound in food with an NH group can be nitrosated to give NVNC. The concentration of most of these may be of no consequence. However, the most important NVNCs in foods appear to be N-nitrosamino acids—mainly N-nitrosoproline (NPRO), N-nitrosothiazolidine-4-carboxylic acid (NTCA) and N-nitrososarcosine (NSAR). The methods reported for the determination of total N-nitroso compounds of foods fall in two categories. In the first category, use is made of the UV photolysis of the compounds to nitrous acid ( $\text{HNO}_2$ ) and calorimetric determination of the liberated nitrite. In the second category, the N-nitroso compounds are cleaved chemically by treatment with HBr and the liberated nitrosyl bromide or nitric oxide (NO) is then measured colorimetrically or by using a chemiluminescence detector.

The most abundant of all the NVNCs are the N-nitrosoamino acids of which NPRO is the most common. Also NPRO is believed to be one of the precursors of NPYR in fried bacon. Most of the methods for the determination of N-nitrosoamino acids in foods are based on GLC-TEA analysis of suitably volatilised derivatives and a few use HPLC-TEA technique. The separation of N-nitrosamino acid anions can be achieved by ion-exchange or reverse phase HPLC. The GLC-TEA technique offers some advantages.

Of all the NVNCs, the nitrosamides have proven to be the most difficult to determine in foods. Since most N-nitrosamides are nonvolatile and thermally unstable they cannot be analysed by GLC and MS. Some recent modifications in TEA instrument have given encouraging results and allowed sensitive detection of some of the N-nitrosamides by GLC-TEA. There is, however, great deal of scope for developing sensitive methodologies for determination of N-nitrosamides which are direct acting carcinogens. Determination of N-nitrosopeptides is still in exploratory stages. Possible contaminants of food e.g. N-nitroso derivatives of some pesticides and a few indole compounds have been investigated. The formation of N-nitrosoharman and N-nitrosonorharman during cooking of tryptophan-rich foods has been postulated but suitable analytical methods for their determination in foods are not yet available.

As in the case of VNAs the final confirmation of the structure of the NVNCs rests with mass spectrometric techniques. Alternatively NVNCs can be directly analysed by HPLC-MS which would eliminate the need for derivatisation. This

technique will be especially useful for confirmation of NVNCs that are not amenable to derivatisation and are thermolabile and for determination of compounds of unknown structures.

## CONCLUSION

Since the levels and nature of N-nitroso compounds in the environment and their effect on human health are intimately linked, it is important that data from different countries and regions are collected and compared. Analysis of air and water samples, edibles and tobacco from various regions needs to be carried out for nitrosamines and their precursors. Further refinement of methods for qualitative and quantitative analysis of volatile nitrosamines and concerted effort to develop analytical techniques for nonvolatile nitroso compounds is imperative. Systematic work on relative risk posed to human and animal health by dietary intake of nitrosatable precursors needs to be intensified.

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